Etiology of Acromegaly: A Molecular Biological Approach

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Acromegalic patients with *gsp* oncogene show a progression of the disease similar to that characterizing patients without this alteration. The low rate of tumor growth has been confirmed by long-term (up to 10 years) follow-up evaluation; in this study, no recurrence of adenoma with the *gsp* oncogene is reported. These data correlate well with the poor morphological evidence for cell replication in tumors expressing *gsp* mutations. In fact, from morphological studies these adenomas appear to be made up of densely granulated cells with a well-developed secretory apparatus, without detectable mitoses. Therefore, according to morphological parameters, tumors expressing the *gsp* oncogene seem to belong to the so-called densely granulated adenomas, which are generally considered less invasive growth hormone (GH)-secreting adenomas. Moreover, these patients are reported to be extremely sensitive to the inhibitory action of somatostatin, which reduces GH release in normal and tumoral somatotropes by inhibiting cyclic adenosine monophosphate (cAMP) production. This suggests that the expression of oncogenic mutations might be partially counteracted in vivo by inhibitory agents. Further studies are required to determine which activating mutations other than *gsp* may occur, whether these mutations occur as an early or late step, and what the role of tumor suppressor gene is in GH-secreting adenoma formation. Finally, the reason that almost all GH-secreting tumors histologically remain benign still eludes investigators.

IN RECENT YEARS, molecular biological approaches have provided important insights into the pathogenesis of pituitary adenomas, strongly supporting the existence of a primary pituitary defect in these tumors. X-chromosomal inactivation analysis demonstrates that the majority of these tumors are monoclonal and points to the existence of genomic mutations in the progenitor cell.[1,2] Although these data strongly support the view that pituitary adenomas arise as a consequence of intrinsic cellular alterations, it is likely that promoting agents such as hypothalamic neurohormones and growth factors may be required for the selective growth of genetically altered cells.[3,4]

GENETIC ALTERATIONS IN GROWTH HORMONE–SECRETING ADENOMAS

Genetic alterations have been implicated in the initiation of a variety of human benign and malignant tumors. Alterations that include either loss of tumor suppressor genes or activation of proto-oncogenes have been identified in pituitary adenomas[5-7] (Table 1). Tumor suppressor genes may be converted to recessive oncogenes, since they require mutations involving both copies of gene to inactivate production of the antiproliferative protein. Therefore, two genetic alterations are generally necessary to disrupt cell turnover regulation: mutation of the tumor suppressor gene and loss of the normal allele. The identification of tumor suppressor genes has come mainly from studies of loss of heterozygosity where regions of chromosomal deletion are demonstrated by comparing genomic and tumor DNA. This approach has been recently extended to human pituitary adenomas. By studying loss of heterozygosity, allelic deletions involving chromosome 11 have been identified in approximately 20% of sporadic pituitary tumors of different types, including somatotropinomas, whereas deletions on other autosomes were infrequently observed.[7] This is in agreement with the notion that this region contains the MEN 1 gene, and deletions at 11q13 have been identified in other sporadic endocrine tumors that are included in this syndrome together with pituitary tumors such as parathyroid tumors.

Screening for genetic abnormalities of other anti-oncogenes in pituitary tumors yielded negative results. In fact, alterations were found neither in the p53 gene, which encodes a 53-kd protein with antiproliferative action, nor in the retinoblastoma gene, another tumor suppressor gene.[5,6]

Dominantly acting oncogenes arise by mutation, rearrangement, or amplification of a single copy of a normal

Table 1. Genetic Abnormalities in Pituitary Tumors

Abnormality	Frequency*
Inactivation of tumor suppressor genes	
Deletion	
Chromosome 11	16/88 all subtypes
Other chromosome	0-3/88 all subtypes
Retinoblastoma gene	0/18 all subtypes
Mutation	
p53	0/50 all subtypes
	0/5 pituitary carcinoma metastases
Activation of cellular oncogenes	
Mutation	
Gs α-subunit	40% somatotropinomas
	2/21 nonfunctioning adenomas
ras	1/87 all subtypes
	3/5 pituitary carcinoma metastases
Protein kinase C	4/4 invasive subtypes
Overexpression	
hst	2/5 prolactinomas
fos	27/33 all subtypes
jun	21/33 all subtypes
myc	1/33 all subtypes

*Positive/tested.

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cellular gene. In the large majority of pituitary tumors, evidence of genetic abnormalities of several recognized cellular oncogenes is still lacking. Activation of the *ras* oncogene has been identified in a very aggressive prolactinoma and in pituitary carcinoma metastases.[8] No evidence of amplification or rearrangement of several other recognized cellular oncogenes was found. Accordingly, although *fos, jun,* and *myc* oncoprotein immunoreactivity has been detected in the majority of pituitary tumors, no correlation with tumoral growth and cellular proliferation was found.[9]

The possible presence of growth hormone (GH) gene abnormalities in somatotropinomas has recently been ruled out.[5]

At present, the genetic abnormality most frequently observed in pituitary tumors concerns the gene encoding the α-subunit of Gs, the protein that stimulates adenylyl cyclase.[10-14] All G proteins become active on binding guanosine triphosphate (GTP), but their intrinsic ability to hydrolize GTP eventually converts them to the inactive guanosine diphosphate (GDP)-bound form.[15-17] G proteins are composed of three subunits: α-subunit, which contains the GDP/GTP binding site and has intrinsic GTPase activity, and β- and γ-subunits. The receptor occupancy catalyzes the exchange of GTP for GDP on the α-subunit, causing dissociation of the α-subunit from the βγ-dimer. The duration of subunit separation is determined by the rate of α-subunit–mediated hydrolysis of GTP. The functional specificity of each G protein is due to the α-subunit, which differs from one G protein to another; the α-subunit of Gs is involved in the stimulation of adenylyl cyclase activity.

In somatotropes the intracellular levels of cyclic adenosine monophosphate (cAMP) are attentively controlled by GH-releasing hormone and somatostatin, which regulate adenylyl cyclase activity by activating Gs and the inhibitory Gi, respectively. Mutations of the α-subunit of Gs were first identified in a subset of GH-secreting adenomas: analysis of DNA from these tumors by the polymerase chain reaction and direct sequencing revealed single amino acid substitutions within the Gs α-subunit gene. Mutations replaced Arg 201 with either Cys or His, or Gln 227 with either Arg or Leu.[11] The presence of the same mutations was subsequently confirmed in other series of somatotropinomas using allele-specific hybridization. Genomic DNA from peripheral blood cells contained only wild-type sequence, in keeping with the view of a somatic origin of genetic mutations. When mutant α-subunit was transfected into α-subunit–deficient S49 cyc– cells, a 30-fold decrease in intrinsic GTPase activity was observed.[11]

Therefore, mutations stabilize Gs in its active conformation, thus leading to constitutive activation of adenylyl cyclase and high levels of cAMP accumulation and GH release. Since in pituitary cells cAMP has a proliferative role, it was proposed that in these cells the gene encoding Gs α-subunit can be converted to an oncogene designated *gsp* for Gs protein.[13] Subsequently, *gsp* oncogene has also been identified in approximately 20% of nonfunctioning pituitary adenomas and in other endocrine disorders such as thyroid functioning adenomas, differentiated cancers, and the McCune-Albright syndrome.[14,18,19]

IN VIVO AND IN VITRO PHENOTYPE OF SOMATOTROPINOMAS EXPRESSING *GSP* ONCOGENE

The in vitro and in vivo phenotypes of tumors carrying the *gsp* oncogene were consistent with the constitutive activation of Gs. In fact, this subset of GH-secreting adenomas was characterized by high levels of in vitro GH release, intracellular cAMP, and membrane adenylyl cyclase activity. The enzyme activity was not further stimulated by specific (such as GH-releasing hormone and vasoactive intestinal peptide) and aspecific (such as GTP and fluoride) agents.[10] The hypersecretory state of tumors expressing the *gsp* oncogene was confirmed by the presence of morphological characteristics in the removed tumors at electron microscopy examination. In fact, these tumors appear to consist of densely granulated cells with a well-developed secretory apparatus.[20]

The in vivo studies indicate no difference in age, sex, clinical features, duration of the disease, or cure rate between patients with and without the *gsp* oncogene.[20] Although no striking difference in circulating GH levels was observed in patients with the *gsp* oncogene, these patients were reported to develop full manifestations of the disease in the presence of very small tumors. These data further confirm that the *gsp* oncogene causes a high rate of secretory activity both in vivo and in vitro. Conversely, no difference in tumor growth was observed between patients with and without the *gsp* oncogene. In fact, acromegalic patients with the *gsp* mutation show a progression of the disease similar to that characterizing patients without this alteration. The low rate of tumor growth has been confirmed by long-term (up to 10 years) follow-up evaluation; in this study, no recurrence of adenoma with the *gsp* oncogene is reported. These data correlate well with the poor morphological evidence of cell replication in tumors expressing *gsp* mutations as documented by the lack of detectable mitoses at electron microscopy. Therefore, according to the morphological parameters, tumors expressing the *gsp* oncogene seem to belong to the so-called densely granulated adenomas, which are generally considered less invasive GH-secreting adenomas.

Patients with *gsp* mutations are generally extremely sensitive to the inhibitory action of somatostatin, which reduces GH release in normal and tumoral somatotropes by inhibiting cAMP production. This suggests that the expression of oncogenic mutations might partially be counteracted in vivo by inhibitory agents.[20]

The in vivo phenotype of patients with the *gsp* oncogene is clearly different from that reported in patients with the *ras* oncogene. In fact, the *ras* oncogene has been reported only in pituitary tumors characterized by unusual invasiveness, such as prolactinoma with frequent recurrences, insensitivity to dopamine, and fatal progression, and pituitary carcinoma metastases.[8]

CONCLUSIONS

Although somatotropinomas are monoclonal in origin, genetic abnormalities have only been identified in about half of these tumors. Abnormalities include both oncogenic mutations involving the Gs α-subunit gene and loss of tumor suppressor gene, probably related to the MEN1-gene. However, further studies are required to determine which activating mutations other than *gsp* may occur in somatotropinomas, whether these mutations occur as an early or late step, and what antiproliferative protein is involved in GH-secreting adenoma formation. Finally, the reason that almost all GH-secreting tumors remain benign histologically still eludes investigators.

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