

CURRENT DATA ON THE ETIOPATHOGENESIS OF PITUITARY ADENOMAS

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REZUMAT

Patogeneza moleculară a afecțiunilor pituitare este complexă și a fost investigată atât pe modele animale cât și în studii pe subiecți umani. Tumorigeneza endocrină (și cea pituitară) are caractere particulare legate de dependența tesuturilor endocrine de factori neurohormonali, care ridică probabilitatea unei activări a mecanismului oncogen și a stimulării sistemelor de reparare a ADN, care sunt predispuși la erori.

Astfel iau naștere două teorii privind geneza tumorală pituitară: stimularea hormonală extrapituitară și leziunea intrinsecă. Ambele teorii sunt importante; geneza tumorală pituitară este un proces seriat, în care alterarea genomică inițială modifică sensibilitatea celulară la diferiți factori externi. Predispoziția ereditară, factorii endocrini și hipotalamici, precum și mutațiile genomice, par să joace toate un rol fiziopatologic în inițierea și progresia adenoamelor pituitare.

Înainte de a trece în revistă contribuția relativă a fiecărei din aceste teorii la dezvoltarea tumorilor pituitare, este important de menționat că creșterea tumorală la nivelul pituitarei este un proces monoclonal. Această observație are o relevanță conceptuală considerabilă și asigură fundalul pe care toate celelalte evenimente fiziopatologice trebuie integrate.

Genele identificate în sindroamele tumorale pituitare familiale afectează transcripția (MEN1) și mesagerii hormonală hipotalamici (CNC1, gsp). Studii asupra disrupției genice murine (Rb, p27, p18) implică gene reglatoare ale ciclului celular, iar câteva dintre acestea au expresia alterată în majoritatea tumorilor pituitare umane (p16, p27). Expresia aberantă a PTTG, un omolog al securinei la mamifere, poate duce la dezagregarea cromozomială, o etapă precoce în procesul de progresie tumorală. Excesul hormonilor hipotalamici trofici duce la hiperplazia pituitară cu exces hormonal, deși rareori se observă alterarea genelor pentru receptorii hormonilor hipotalamici.

ABSTRACT

The molecular pathogenesis of pituitary disorders is complex and has been explored in animal models and human subject studies. The endocrine (and pituitary) tumorigenesis process has particular aspects related to the dependency of the endocrine tissue on neurohormonal factors, that raise the probability of oncogene mechanisms activation and induction of DNA repairing systems that may be predisposed to errors.

Thus the two theories expressing the pituitary tumorigenesis take shape: the extrapituitary hormonal stimulation and the intrinsic lesion. Both theories are important; the pituitary tumorigenesis is a multistep process, in which the initial genomic alteration modifies the cell sensitivity to various external factors. Hereditary predisposition, endocrine and hypothalamic factors and genomic mutations all seem to have a pathophysiologic role in the initiation and progression of pituitary adenomas.

Before considering the relative contribution of each of these theories in the development of the pituitary tumor, it is important to note that the tumor development in the pituitary is a monoclonal process. This observation has considerable conceptual relevance and ensures the background on which all other pathophysiologic events must be integrated.

Genes identified for familial pituitary tumor syndromes affect transcription (MEN1) and hypothalamic hormone signaling (CNC1, gsp). Murine gene-disruption studies (Rb, p27, p18) implicate cell-cycle regulatory genes, some of which have altered expression in most human pituitary tumors (p16, p27). Aberrant expression of PTTG, a mammalian securin homolog, can lead to chromosome missegregation, an early step in multistep pituitary tumor progression. Excess hypothalamic trophic hormones lead to pituitary hyperplasia and hormone excess, whereas alterations in hypothalamic hormone receptor genes are uncommon.

INTRODUCTION

Symptomatic anterior pituitary tumors represent approximately 10% of intracranial neoplasms, and clinically inapparent "incidentalomas" are quite common, discovered in as many as 10 to 20% of unselected autopsies.¹ Despite pituitary tumors are usually benign adenomas, they can cause significant morbidity because of their size and/or inappropriate

expression of pituitary hormones. Mass effects include severe headaches, visual dysfunction, and/or altered hormone expression because of pituitary stalk compression, and can occur whether or not a tumor hypersecretes hormones.

Unique clinical symptoms correspond to the nature of the hormone hypersecretion. Somatotropinomas overexpress GH, causing acromegaly in adults with overgrowth of soft tissues and bone, and an increased risk of hypertension, cardiac disease, and diabetes.² In rare cases, somatotropinomas cause gigantism in prepubertal

patients. Prolactinomas overexpress PRL, usually presenting as amenorrhea and galactorrhea in females, and impotence, hypopituitarism, and mass effects in males. Both sexes are at increased risk of osteoporosis. Prolactinomas are the most common of all pituitary adenomas, occurring with a 3:1 female preponderance.³ Tumors expressing both PRL and GH originate from precursor mammosomatotrophs. Corticotropinomas, responsible for Cushing's disease, produce increased levels of ACTH and lead to adrenal gland overstimulation. Excess cortisol, adrenal androgens, and 11-deoxycorticosterone lead to truncal obesity, protein and muscle wasting, backaches, impaired immunity, cardiovascular complications, osteoporosis, psychiatric disturbances, infertility, and female hirsutism.⁴ Occurring rarely, pure gonadotropinomas cause sexual dysfunction and hypogonadism.⁵ Thyrotropinomas are very rare, and cause a mild increase in T4 levels.⁶

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Thus the two theories expressing the pituitary tumorigenesis take shape: the extrapituitary hormonal stimulation and the intrinsic lesion. Both theories are important; the pituitary tumorigenesis is a multistep process, in which the initial genomic alteration modifies the cell sensibility to various external factors. Hereditary predisposition, endocrine and hypothalamic factors and genomic mutations all seem to have a pathophysiologic role in the initiation and progression of pituitary adenomas.

Before considering the relative contribution of each of these to pituitary tumor development, it is important to acknowledge that tumor development in the pituitary is a monoclonal process. This observation is of considerable conceptual relevance and provides the background on which other pathophysiologic events must be integrated.

One of the most fundamental and historically contentious issues surrounding pituitary tumorigenesis relates to whether transformation in the pituitary is primarily the product of hypothalamic dysfunction or simply the result of an acquired transforming mutation intrinsic to an isolated adenohypophysial cell. The hypothalamic hypothesis suggests that pituitary adenomas arise as the eventual, downstream, and

seemingly passive consequence of excessive trophic influences, emanating from a dysfunctional hypothalamus. Alternatively, the pituitary hypothesis suggests that pituitary adenomas arise as the direct result of an intrinsic pituitary defect, with neoplastic transformation occurring in relative autonomy from hypothalamic trophic influence. Whereas substantial evidence exists in support of both possibilities, the latter concept has been especially favored in view of the lack of peritumoral hyperplasia in association with pituitary adenomas and because many pituitary tumors can be definitively "cured" when completely removed. Neither of these would be expected were hypothalamic overstimulation the dominant tumorigenic mechanism.⁷

TRANSCRIPTION FACTOR REGULATION OF PITUITARY TUMORS

The anterior pituitary integrates stimulatory and inhibitory hypothalamic signals to synthesize and secrete trophic hormones that regulate growth (GH), sexual development and function (LH/FSH, PRL), metabolism (TSH), and stress responses (ACTH). These hormones are respectively produced by five cell types: somatotrophs, gonadotrophs, lactotrophs, thyrotrophs, and corticotrophs. Pituitary development and hormone expression are controlled by precisely timed expression of homeobox transcription factors and hypothalamic regulatory signals. Pituitary dysfunctions arise when some or all of these hormones are deficient or produced in excess.

The five hormone-specific subtypes of anterior pituitary cells are derived from a pluripotent precursor early in embryonic development by exquisitely controlled expression of homeodomain transcription factors.

Because deficiencies in transcription factors lead to decreased pituitary hormone expression, these factors have been screened for altered expression or splicing as causative defects in pituitary tumors. *Pit1* mRNA was increased 2.5- to 5-fold in pituitary adenomas compared to normal pituitary tissue, but the cell type distribution, size, and sequence of the *Pit1* transcripts appeared intact. *Pit1* isoforms with different DNA-binding and transactivation properties are encoded by alternatively spliced *Pit1* mRNAs.⁸ Similar ratios of the *Pit1* and *Pit1b* isoforms were observed in pure GH- or PRL-secreting tumors, suggesting that pituitary tumorigenesis is not associated with altered *Pit1* expression.

Prop1 in the mouse is not expressed beyond embryonic development, and prolonged expression

is tumorigenic in transgenic mice.⁹ However, *Prop1* mRNA was detected in normal human pituitary (n = 4) and pituitary tumors (n = 18) by a nonquantitative RT-PCR study.¹⁰ *Prop1* coding sequences were also normal in the nine tumors examined. Thus, these initial studies suggest that altered *Prop1* expression or sequence does not appear to play a role in pituitary tumorigenesis.

ONCOGENES

Genomic Alterations: Oncogene Activation

Accompanying the realization that the somatic mutation of an isolated adenohypophysial cell is an event requisite to pituitary tumorigenesis, vigorous attempts have been made to identify and characterize the responsible mutation. Of the genomic and cellular alterations known to occur in pituitary adenomas, relatively few appear to involve activating mutations of known oncogenes. To date, activating mutations of only two oncogenes have been reported in pituitary adenomas: *gsp* and H-ras. Whereas the former is encountered with some regularity in somatotrope adenomas and periodically in other pituitary adenoma types, the latter has been identified in only isolated instances.

Mutation in the α subunit of one of the G proteins (Gsa) produces the oncogene *gsp*, which results in constitutive activation of cyclic AMP (cAMP). Reported in 30 to 40% of GH-secreting tumors, the *gsp* oncogene has not been shown to be abnormal in nonfunctioning tumors,¹¹ and no activating mutations of the *Gaq*, *Ga11*, or *Gas* genes were detected in nine TSH-secreting tumors.¹² Pituitary tumor transforming gene (*PTTG*), located on chromosome 5q33, expresses fibroblast growth factor (FGF).

Pituitary tumor transforming gene and FGF have been detected in many subtypes of tumors (GH, PRL, ACTH, TSH [n=1], and nonfunctioning) and correlate with invasiveness.^{13,14} The *ras* oncogene has been identified in invasive and metastatic prolactinomas.¹⁵ Allelic imbalance of the cyclin *D1* gene, located on chromosome 11q13, leads to overexpression of cyclin D1 protein. Such overexpression occurs in both nonfunctioning and GH-secreting tumors, whereas *hst* (heparin-binding secretory transforming gene) is overexpressed in prolactinomas. Bcl-2 (an apoptosis antagonist) and c-myc (a transcription factor) are oncoproteins overexpressed in some tumors.^{15, 16}

PROTEIN KINASE C GENE

Another genomic alteration identified in pituitary adenomas, specifically invasive ones, relates to the

protein kinase C (PKC) family of second messengers. PKC family members are ubiquitous, membrane-bound, intra-cellular kinases whose function is to phosphorylate serine or threonine residues on important substrate proteins. Such kinase activity is thought to govern several important cellular processes, including the transmembrane signaling underlying cell proliferation and differentiation. Altered or aberrant PKC activity has been demonstrated in several human tumors, including pituitary adenomas. In comparison with normal pituitary tissue, increased PKC protein expression was identified in pituitary adenomas. More recently, it has been demonstrated that the specific PKC isoform that is overexpressed in pituitary tumors is PKC- α . Interestingly, this particular PKC isoform has been favored as the isoform that mediates the mitogenic functions of PKC. Not only was PKC- α found to be overexpressed in pituitary adenomas, but invasive adenomas also exhibited a conserved point mutation of the PKC- α gene.⁷

TUMOR-SUPPRESSOR GENES

Farrell et al (1999) have described three mechanisms for inactivating tumor suppressor gene function.¹⁷ These include loss of heterozygosity (LOH) with the retained allele being mutated, homozygous deletion of both alleles, or methylation of CpG islands. When small DNA regions (CpG islands) in the 5'-promoter region of genes are methylated, then transcription is repressed. In the case of tumor suppressor genes, the result is functional silencing of a normal gene.¹⁸

The retinoblastoma gene (**Rb - 13q14**) remains the prototypical example of this class of genes. Beyond its role in the development of familial retinoblastoma and various other malignancies and its overall contribution to cell-cycle regulatory control, studies of the Rb gene added a new dimension to the very concept of human cancer, illuminating recessive aspects of the process and the oncogenic consequences that accompany loss of protective genomic elements. The implication that the Rb gene might be involved in pituitary tumorigenesis had a somewhat serendipitous beginning. Transgenic mice in which one of the two germline Rb alleles had been deactivated failed to develop retinoblastomas as anticipated; instead they developed large, high-grade, invasive pituitary tumors. On further analysis, these tumors were shown to have lost the remaining normal Rb allele, convincingly implicating a second Rb "hit" as the basis for pituitary tumor development in this model. All of these tumors were found to be corticotropic in nature, immunoreactive for ACTH, and of pars intermedia origin.

Prompted by the provocative nature of these findings, a number of recent studies have sought to determine the relevance of Rb mutations in human pituitary tumors. In the first report, none of 18 informative pituitary tumors exhibited allelic Rb loss.¹⁹ This was further confirmed in a study of 30 informative pituitary adenomas where in none was found to exhibit loss of heterozygosity (LOH) at the Rb gene locus.²⁰ In another study, however, LOH was found at the Rb locus in all of seven pituitary carcinomas, including their metastatic deposits, and in all of six highly invasive pituitary adenomas.²¹ The significance of these latter observations vis-à-vis Rb gene mutations, however, was undermined by the finding of Rb protein in tumors exhibiting LOH at the Rb locus. In reconciling the apparent discordance between Rb gene and protein status, together with the two previous studies that excluded Rb mutations within pituitary tumors, the conclusion was made that another putative tumor-suppressor gene, one present on 13q14 but distinct from Rb, must be involved in pituitary tumor progression.

p53. The p53 checkpoint protein prevents cell cycle progression when DNA is damaged, blocking G1/S or G2/M progression and inducing apoptosis. Whereas p53 is the most commonly mutated gene found in human tumors, it does not appear to sustain mutations in pituitary adenomas. Alterations in the commonly mutated exons 5 through 8 were not detected in a large series of pituitary adenomas or carcinomas.^{22,23} Mutant p53 forms accumulate in the nucleus, with a longer half-life than wild-type p53, although the correlation is not absolute. Whereas some investigators reported p53 immunopositive noninvasive adenomas, others²⁴ found a clear correlation between p53 nuclear immunopositivity (reflecting aberrant p53 protein) and tumor aggressiveness, with p53 expressed in 100% of carcinomas, in 15% of invasive adenomas, and none of 37 noninvasive adenomas.

nm23. The purine-binding factor *nm23* is a tumor suppressor in rodents with decreased expression in highly metastatic melanoma cell lines. The two *nm23* isoforms, H1 and H2, were screened for altered expression in 22 pituitary tumors. Invasive tumors had decreased expression of the H2 isoform, compared to benign adenomas.²⁵

MEN1 Tumor-Suppressor Gene. Genetic predisposition to pituitary tumor development is restricted to a single and uncommon condition, the MEN1 syndrome. This autosomal-dominant condition is characterized by the simultaneous development of tumors involving the parathyroid

glands, pancreatic islet cells, and the pituitary. A variably penetrant condition, only 25% of patients develop pituitary tumors, the majority of which are macroadenomas associated primarily with GH and/or PRL hypersecretion.^{26,27} Approximately 3% of all pituitary adenomas occur in the context of MEN1. The nature of the genetic defect in MEN1 has recently been identified and involves allelic loss of a putative tumor-suppressor gene at the 11q13 locus.^{28,29} In its recessive behavior, the MEN1 gene is typical of a tumor-suppressor gene, with susceptible individuals inheriting a germline mutation of one of the two 11q13 alleles. Subsequent spontaneous mutation, inactivation, or deletion of the remaining normal 11q13 locus in susceptible endocrine tissues ultimately leads to tumor formation in the involved tissue.

Once believed to be a genetic defect that accounted for pituitary adenomas occurring exclusively in the context of MEN1, several studies have also demonstrated loss of the 11q13 locus in seemingly sporadic pituitary adenomas. In the earliest of these, allelic deletions of 11q13 were found in two of three sporadic prolactinomas. Subsequently, 4 of 12 sporadic GH cell adenomas were found to have deletions involving the 11q13 locus. More recently, allelic deletions of chromosome 11 were found in 18% of pituitary adenomas of all major types. Collectively, these data suggest that the 11q13 locus is the site of an important tumor-suppressor gene, the inactivation of which may be of pathogenic relevance to the development of both sporadic and MEN1-related pituitary adenomas.

Cdk inhibitors. Cyclin-dependent kinases (Cdk's) tightly control progression of the eukaryotic cell cycle through G1 to S phase.³⁰ The Cdk inhibitors block cell cycle progression and act as tumor suppressors, restraining cell division. Their loss by homozygous inactivation or failed expression results in progression through the cell cycle. Cdk inhibitors (p16Ink4a, p15Ink4b, p18Ink4c, p19Ink4d) block the interaction of CDK4 and CDK6 kinases with cyclin D, and Cip/Kip proteins (p21Cip1, p27Kip1, p57Kip2) bind to cyclin-CDK complexes and inhibit their activation or kinase activity.

p27. Mice disrupted for *p27* develop multi-organ hyperplasia and frequent tumors of POMC-positive cells of the pituitary intermediate lobe, and cell divisions increase in organs that normally express *p27*.³¹ In this case, *p27* behaves as a tumor suppressor gene both in the animal model and in human sporadic pituitary tumors. Immunodetectable *p27* protein is underexpressed or absent in most human pituitary tumors of all subtypes and is undetectable in pituitary

carcinomas.³² Because *p27* mutations were excluded by SSCP analysis, it appears that *p27* expression is regulated by posttranscriptional and posttranslational mechanisms, including ubiquitin-dependent protein degradation.³³

p18. Mice disrupted for the cyclin inhibitor *p18Ink4c* have widespread organomegaly and pituitary hyperplasia and develop intermediate lobe tumors at an advanced age.³⁴ Because GH levels are normal and IGF-1 is only slightly elevated in these animals, tissue overgrowth is most likely caused by an intrinsic defect in *p18*, rather than from endocrine effects of pituitary hyperplasia. Mice that are doubly disrupted for *p18* and *p27* die within 3 months and have greatly accelerated pituitary tumorigenesis, demonstrating that these genes act synergistically.

p16. *p16Ink4a*, encoded by the *CDKN2A* gene on chromosome 9p21, maintains Rb in an unphosphorylated, active state by blocking CDK4. *p16* is inactivated in several human tumor cell lines and in primary tumors, and *p16* was undetectable by Western blot analysis of 25 pituitary tumors.³⁵ Mutations were not detected, but three of these tumors had homozygous *p16* gene deletions. Loss of *p16* protein correlated with decreased or undetectable mRNA, but in contrast to other tumor types in which *p16* LOH or homozygous deletions are common, pituitary adenomas more frequently inactivate this gene by methylation. The reintroduction of an inducible *p16* gene into AtT20 murine corticotropinoma cells caused reversible growth inhibition and G1 arrest,³⁶ supporting a role for this gene in pituitary tumorigenesis.

GROWTH FACTORS AND CYTOKINES

Estrogen. Estrogen is a mitogen for normal and transformed lactotrophs and gonadotrophs and is a ligand for the estrogen receptor (ER). Pharmacologic doses of estrogen induce rat lactotroph hyperplasia and adenomas,³⁷ and prenatal exposure to diethylstilbestrol causes an 11-fold increase in prolactinomas in female mice offspring. The female preponderance of prolactinomas and their increased size during pregnancy may be caused by high estradiol levels, especially because prolactinomas most strongly express estrogen receptors. Besides stimulating the prolactin promoter, estrogen activates *PTTG* and *TGF α* expression, two genes implicated in pituitary tumorigenesis. ER is encoded by two genes: *ER α* , expressed in 70 to 100% of prolactinomas, and *ER β* , detectable in 60% of tumors. Estradiol binding leads to ER dimerization and activation of estrogen-

responsive genes, with a stronger estrogen response caused by *ER α* than *ER β* .³⁸ Alternatively spliced *ER α* mRNAs encode isoforms with altered responsiveness to estrogens and antiestrogens, suggesting an attractive mechanism for tumorigenesis. In particular, exon 2 or exon 5 deletions produce stimulatory or ligand-independent isoforms, respectively. Exon 2- and exon 5-deleted transcripts were detected in nearly all prolactinomas. In contrast, exon 3 or exon 7 deletions encode dominant negative *ER α* isoforms lacking DNA binding or transactivation functions, respectively. Exon 7 deletions were much less common in prolactinomas, although the relative proportions of mutant and full-length *ER α* cDNAs were not determined. *ER β* is not strongly activating by itself and is not expressed in all prolactinomas, but nonetheless interacts with exon 5-deleted *ER α* to increase expression of an estrogen response element (ERE)-controlled luciferase reporter gene.³⁸

GFG. Mammals transcribe an antisense mRNA, termed GFG, that overlaps the 3' exon and 3' UTR of bFGF. Levels of GFG and bFGF transcripts are inversely proportional in some pituitary adenomas. Initially, it was postulated that the GFG transcript inhibits bFGF expression by an antisense mechanism, but the open reading frame encoded by GFG is required for inhibition of bFGF expression. Interestingly, the anti-bFGF effects of GFG are uncoupled, because it inhibits proliferation but stimulates PRL expression in GH4C1 cells.³⁹

NGF. Nerve growth factor, NGF, functions in growth, differentiation, and neuronal survival through binding to tyrosine kinase receptors. In the anterior pituitary, cells of the mammosomatotroph lineage express NGF and its receptors. Female transgenic mice, in which NGF transcription is driven by the rat prolactin promoter, develop lactotroph hyperplasia without adenomas despite having pituitary glands 10 to 100 times larger than normal.⁴⁰

β FGF. Basic fibroblast growth factor, or bFGF, a potent mitogen for neuroectoderm cells in vitro, stimulates angiogenesis in vivo, and its expression is highest in the pituitary and brain. Although lacking a classic aminoterminal signal sequence, bFGF is secreted but normally undetectable in human serum. However, in 7 of 12 patients with MEN I and prolactinomas, immunoreactive bFGF was detectable before surgery or dopamine agonist treatment, and decreased after surgery or bromocriptine. Elevated plasma bFGF was not detectable in three patients with sporadic macroprolactinomas, but intratumoral bFGF expression correlated positively with *PTTG* oncogene expression in seven sporadic prolactinomas.³⁷ Synthesis and secretion of bFGF are induced in NIH 3T3 cells

overexpressing the human PTTG proto-oncogene.

TGF α . Transforming growth factor- α , TGF α , a mitogenic protein expressed in several adult tissues including lactotrophs, is secreted in retrovirally transformed cells. In a model of estrogen-induced rat pituitary tumors, pituitary TGF α mRNA levels increase before initiation of lactotroph hyperplasia.⁴¹ TGF α transgenic female mice (with transgene expression driven by the rat PRL promoter) developed lactotroph hyperplasia by 6 months and prolactinomas by 12 months, and transgenic males did not develop pituitary disease. TGF α did not induce tumors of other pituitary cell subtypes, indicating a specific role in prolactinoma tumorigenesis, likely potentiated *in vivo* by estrogen.

HORMONE RECEPTORS

DRD2. The dopamine D2 receptor, DRD2, mediates dopamine inhibition of PRL transcription and secretion and lactotroph growth.⁴² Pituitary stalk compression, neovascularization, or gene disruption blocks this inhibition and results in lactotroph hyperplasia, hyperprolactinemia, and prolactinomas in DRD2-disrupted mice. Two possibilities were suggested to explain the prolonged lactotroph hyperplasia (17–18 months) that preceded adenoma development in female, but not male DRD2 mice, which only developed multifocal microprolactinomas.

One explanation is that preventing dopamine inhibition allowed an increased pool of lactotrophs to acquire initiating tumorigenic changes, in gender-specific tumorigenic pathways. Alternatively, pituitary hyperplasia might not be a prerequisite for tumorigenesis. Reduced dopamine receptor expression correlates clinically with bromocriptine resistance in prolactinomas.²⁷ Otherwise, the DRD2 gene is not implicated in human pituitary tumors, because no pathogenic sequence changes were detected in the D2 receptor in 46 prolactinomas and 19 mixed GH/PRL adenomas.⁴³

Somatostatin receptors. Somatostatin membrane receptors are encoded by SSTR1–5. GH secretion but not transcription is mediated by SSTRs, primarily by SSTR2 and SSTR5. Because some patients display resistance to somatostatin analog therapy, inactivating mutations in the SSTR2 and SSTR5 genes were sought. However, these are uncommonly encountered, at least in the small number of samples screened.⁴⁴

TRH-R. Hypothalamic TRH stimulates TRH release from thyrotrophs and PRL release from lactotrophs. The pituitary G-protein coupled TRH receptor mediates these effects. Somatic activating mutations in the TRH-R gene were excluded in 50 pituitary adenomas (including GH, PRL, TSH, and

NF subtypes). However, a mutation in the ligand-binding domain of TRb was detected in a TSHoma resistant to thyroid hormone.⁴⁵

GHRH-R. The GHRH receptor, GHRH-R, is a seven-transmembrane G-protein-coupled receptor (GPCR). Because GPCR mutations have been observed in other endocrine neoplasias.⁴⁶ and CREB phosphorylation occurs in somatotropinomas irrespective of the *gsp* mutation, activating mutations in the GRHR-R gene were sought to explain constitutive activation of the cAMP signaling pathway. By combining direct sequence analysis and functional tests, GHRH-R activating mutations have been excluded in a total of 80 somatotropinomas.⁴⁶

GnRH-R. The GnRH receptor, a member of the seven-transmembrane domain GPCR family, signals through the G-protein, Gq. Constitutive activation of this pathway through expression of a mutated Gq protein transforms NIH 3T3 cells. Therefore, it seemed likely that the GnRH signaling pathway could be constitutively activated in gonadotropinomas, but the GnRH-R gene sequence was normal in 10 tumors. It has been suggested that activating mutations in GPCRs are not commonly identified, because they have no *in vivo* phenotype owing to receptor down-regulation.^{47–49}

ANGIOGENESIS

Angiogenesis is another mechanism that contributes to tumor invasion, and it may be involved in more aggressive tumors. Several recent reports by Turner and her colleagues have begun to clarify the role of angiogenesis in pituitary tumors. In one study of 160 tumors (GH, PRL, ACTH, and nonfunctioning), microvascular density was not associated with tumor recurrence, although in other studies invasive and large prolactinomas were more vascular than smaller tumors.¹⁶ One possible explanation may be that matrix metalloproteinase 9 expression was greater in invasive prolactinomas and pituitary carcinomas. This relation to size and vascularity was not observed in GH-secreting tumors.

HYPOTHALAMIC AND ENDOCRINE FACTORS

Whereas the demonstration that pituitary adenomas are monoclonal derivatives of a single transformed adenohypophysial cell does conform well to existing paradigms of human tumorigenesis, it should not be interpreted as somehow exonerating hypothalamic influences of a role in pituitary tumor development. On the contrary, the culpability of hypothalamic hormones in pituitary tumorigenesis

continues to gain strength, and there has been renewed interest in integrating a role for these hormones in the current multistep monoclonal model. The class of hypothalamic hormones at issue are the hypothalamic hypophysiotropic hormones, which primarily include GHRH, somatostatin (SRIF), CRH, TRH, gonadotropin-releasing hormone (GnRH), and dopamine. Produced in hypothalamic nuclei, descending via the portal circulation, and binding to specific membrane receptors on their respective adeno-hypophysial target cells, these hormones govern the secretory and proliferative activity of each of the principal pituitary cell types. In logical extension of their physiologic trophic activities has been the implication that aberrant activity of these regulatory hormones in the form of excess stimulation or deficient inhibition may contribute to the genesis and/or progression of pituitary adenomas. For example, in states of pathologic GHRH excess, as occurs with rare GHRH-producing tumors (pancreatic endocrine tumors, carcinoids, pheochromocytomas, and hypothalamic hamartomas/gangliocytomas), chronic GHRH stimulation leads to hyperplasia of pituitary somatotropes, GH hypersecretion, and clinical acromegaly. Depending on the duration of exposure to the excess GHRH, progression from somatotrope hyperplasia to adenomatous transformation has been documented in some, but not all instances.⁷

It should be clear that despite the monoclonal constitution of pituitary adenomas, a potential role of hypothalamic hormones in their genesis and/or progression is not easily dismissed.

A recurring theme, one borne from both experimental study and clinical observation, relates to the possible predisposing, promoting, or perhaps even the inductive effect of certain altered endocrine states to the development of pituitary adenomas. Of particular relevance are those states of target-gland failure wherein the pituitary is no longer subject to negative feedback effects imposed by target-gland hormones. For example, within the pituitary glands of patients with Addison disease and primary hypothyroidism of long duration, the respective frequencies of corticotrope and thyrotrope "tumorlets" was higher than that observed in control individuals. Admittedly, only a loose correlation, but stronger still, and of greater clinical concern is the behavior of corticotrope adenomas and thyrotrope adenomas in the setting of bilateral adrenalectomy (Nelson syndrome) and prior thyroidectomy, respectively. That such tumors tend to be notoriously more aggressive than those having an intact pituitary-target gland axis emphasizes the potential importance of negative-feedback inhibition in modulating the behavior and progression of these neoplasms.

CONCLUSIONS

Early changes leading to pituitary tumorigenesis involve both intrinsic alterations in pituicytes and altered availability of regulatory factors. Familial pituitary tumor studies have identified three genes that predispose to pituitary tumorigenesis and implicate important regulatory pathways.

Tumor suppressor genes that regulate the cell cycle have been implicated in mice with disruptions of *Rb*, *p27* or *p18* who develop pituitary tumors; doubly disrupted animals have enhanced tumorigenesis, suggesting that these genes act synergistically. Whereas active hypophosphorylated Rb protein is detected in human pituitary tumors, other tumor suppressor gene products that regulate Rb phosphorylation have been implicated, either by their decreased expression in human pituitary tumors or their effects in gene-disrupted mice. Decreased p16 and p27 expression is a universal finding in human pituitary tumors, invoking gene methylation and proteolytic pathways in pituitary tumorigenesis.

In contrast to animal models, increased hypothalamic trophic hormone expression can lead to pituitary hyperplasia in human subjects but rarely adenoma formation. Despite extensive searches, mutations in pituitary hormone receptor genes (*GHRH-R*, *GnRH-R*, *TRH-R*, *DRD2*, *SSTR2*, *SSTR5*) are rarely observed in human pituitary tumors. Mitogenic stimulatory pathways involving estrogen, bFGF and TGF α play an important role in pituitary tumor initiation. *PTTG* expression is increased in all pituitary adenoma subtypes and is induced by estradiol, further inducing β FGF synthesis and secretion and promoting angiogenesis. *PTTG* functions as a securing regulating chromosome separation, and evidence is presented supporting the notion that aberrant *PTTG1* expression leads to chromosome missegregation, a link in the multistep progression of pituitary tumors.

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