Molecular genetics of the cAMP-dependent protein kinase pathway and of sporadic pituitary tumorigenesis

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Pituitary tumors are among the most common human neoplasms. Although these common lesions rarely become clinically manifest and they are almost never malignant, they are the cause of significant morbidity in affected patients. The genetic causes of common pituitary tumors remain for the most part unknown; progress has been limited to the elucidation of the molecular etiology of four genetic syndromes predisposing to pituitary neoplasias: McCune-Albright syndrome, multiple endocrine neoplasia type 1, Carney complex and, most recently, familial acromegaly and prolactinomas and other tumors caused by mutations in the GNAS, menin, PRKAR1A, AIP, and p27 (CDKN1B) genes, respectively. Intense molecular studies of sporadic pituitary tumors from patients with negative family histories and no other neoplasms have yielded interesting findings with abnormalities in growth factor expression and cell cycle control dysregulation. To add to the difficulties in understanding pituitary tumorigenesis in man, good murine models of these neoplasms simply do not exist: pituitary tumors are common in rodents, but their histologic origin (mostly from the intermediate lobe), age of presentation (late in murine life) and clinical course make them hardly models of their human counterparts. The present report reviews the clinical and molecular genetics of the cAMP-dependent protein kinase pathway in human pituitary tumors; it also reviews briefly other pathways that have been involved in sporadic pituitary neoplasms. At the end, we attempt a unifying hypothesis for pituitary tumorigenesis, taking into account data that are also discussed elsewhere in this issue.

INTRODUCTION

Pituitary adenomas can occur in a familial setting in multiple endocrine neoplasia type 1 (MEN 1) (1), Carney complex (CNC) (2) and in the context of isolated, autosomal dominant acromegaly or gigantism, and, less frequently, the context of a familial predisposition to the development of other pituitary tumors (3). Another genetic, but not inherited, disorder associated with growth hormone (GH) and prolactin (PRL)-producing pituitary tumors is McCune-Albright syndrome (MAS) (4).

MEN 1 is caused by an inactivating mutation in the menin gene on chromosome 11q13 (5). The clinical presentation of MEN 1, mouse models of the disease and other functions have been extensively presented elsewhere (6–10) and in an accompanying manuscript in this issue (10).

CNC, a rare condition, has been described in about 500 people to date (11) and is caused (in more than 60% of the patients that meet diagnostic criteria) by inactivating mutations in the gene encoding for the protein kinase A (PKA) type 1A regulatory (R1α) subunit (PRKAR1A) (12). PRKAR1A, like menin, acts as a tumor suppressor gene in affected tissues and losses of its normal allele at chromosomal region 17q22–24 are present in pituitary tumors associated with the condition (13). A second, as yet uncharacterized, locus at 2p16 has also been implicated in some families (14,15). Acromegaly in CNC is characterized by a slow, progressive course. The mean age of acromegaly was 35.8 years in the cohort of patients that we recently reported (2,16). It is interesting that in many of these patients clinically significant acromegaly did not become apparent until after they were operated for their Cushing syndrome: 72% of these patients

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had at the same time primary pigmented nodular adrenocortical disease (PPNAD). A change in clinical phenotype in a patient that has concurrently Cushing syndrome and acromegaly is not unexpected given the known relationship between GH and cortisol metabolism (2), but the phenomenon has not been studied in detail in CNC or patients with similar conditions (i.e. MAS).

Among the patients with CNC and acromegaly that were operated for their pituitary tumors, there have been at least four to date who had evidence of GH- and PRL-producing cell hyperplasia on their pituitary histopathology: their GH-producing cells stained positive for PRL and occasionally other pituitary hormones. Staining for a-subunit, b-TSH and b-LH was also present in diffusely and rarely present cells of some adenomas and within foci of normal cells entrapped within the tumors. On the other hand, ACTH and FSH staining, when obtained, could only be seen in foci of normal cells entrapped within the tumors or the hyperplasia (2,17). Multifocal somatomammotrophic cell hyperplasia does not appear to be the case in MEN 1 pituitaries (16). Another indication for the relatively high frequency of pituitary hyperplasia in CNC patients is the fact that clinically evident acromegaly is a relatively infrequent manifestation of the disease, while biochemical abnormalities such as increases of GH, PRL and insulin-like growth factor 1 (IGF1) levels may be present in up to 79% of patients (13,17,18).

Mouse models of R1α deficiency have been created but they failed to reproduce a specific or a significant pituitary phenotype. Three mouse models of prkar1a deficiency have been developed (19–21) without any consistent pituitary abnormalities. A transgenic mouse bearing an antisense construct of the mouse prkar1a exon 2 (X2AS) developed multiple endocrine abnormalities in parallel to some of the features of CNC but there were no significant pituitary abnormalities (see below). Similarly, there were no pituitary lesions in the two prkar1a heterozygote knock-out (KO) models that have been reported (20,21). The pituitary glands of older prkar1a X2AS and heterozygote KO mice were examined recently (22): only mild abnormalities were seen, although an excess of GH-producing cells was confirmed (23). In the absence of any tumors or other structural abnormalities, a cross between the transgenic metallothionine (MT)-driven GH-releasing hormone (GHRH)-overexpressing acromegalic mouse (24) and the prkar1a heterozygote KO mouse model was developed in our laboratory (25). Preliminary analysis showed that in the presence of a tumorigenic, proliferative signal such as that of GHRH, prkar1a deficiency was associated with more significant somatomammotrophic hyperplasia (26).

MAS, caused by mosaicism for a mutation in the GNAS oncogene, is characterized by polyostotic fibrous dysplasia (PFD), pigmented skin lesions and overactivity of almost all endocrine glands including the pituitary (4). Up to a fifth of MAS patients have GH excess but only few develop detectable pituitary tumors, a situation that is similar to that in CNC (27,28). The pituitary gland in MAS patients may show GH- and PRL-producing cell hyperplasia, as in CNC patients (29). The consequences of hypersomatotropinemia in MAS can be, however, grave since PFD seems to be getting worse in the presence of elevated GH levels (30) which have also been implicated in sarcomatous transformation of these bone tumors (31). GH-producing tumors in MAS show a consistent but inadequate response to treatment with cabergoline, and an intermediate response to long-acting octreotide; GH-receptor antagonists have recently been proposed as effective medical agents for inoperable MAS pituitary tumors or for simple hypersomatotropinemia without a visible adenoma (32,33).

Most recently, heterozygous germline mutations were reported in the aryl hydrocarbon receptor interacting protein (AIP) gene in families of European descent with predisposition to develop mainly GH-producing pituitary tumors (34). AIP involvement in pituitary tumorigenesis is reviewed extensively in this issue by Karhu and Aaltonen (10). Familial isolated pituitary adenomas (FIPA) also occur, albeit rarely outside of MEN1, CNC and AIP mutations (35–37). A recent study from a large international consortium studied 64 families with FIPA (36). Isolated GH- and PRL-producing tumors were the most common among them, whereas familial non-secreting tumors were of relatively low incidence. Familial corticotropin (ACTH)-producing tumors were rare; indeed, familial Cushing disease has only been reported rarely in the literature (37), outside of MEN 1 (38). Nevertheless, these studies indicate that other genes may cause familial pituitary tumors in individual kindreds, and are likely to play a role interacting with the cAMP-dependent protein kinase pathway. Recently, a family with pituitary and other tumors was reported in association with a single germline p27 mutation (39). This is an interesting report because of the known interactions between PKA and cyclins (40).

Camp-dependent protein kinase signaling and pituitary tumors

Pituitary tumors are an integral part of MAS (4), as outlined above. GNAS is a ubiquitously expressed gene that maps on chromosome 20q13 and codes for the stimulatory G protein (Gαs) that is required for the activation of adenyl cyclase and generation of cAMP in many cell types, including most of the pituitary cells. GNAS was found mutated in sporadic GH-producing tumors, a finding that heralded the identification of the same genetic defect in MAS: amino acid substitutions at Arg 201 (and rarely at Gln 227) (41). It should be noted that these tumors were characterized by high adenyl cyclase activity and cAMP levels (41). When transfected in vitro mutant GNAS cells showed up to a 30-fold decrease in the rate of subunit-mediated hydrolysis of GTP to GDP, a step that is required for the reassembly of the G-protein β heterotrimer and signals the end of the activation (42).

The identification of GNAS as the gene responsible for a large number of sporadic GH-producing pituitary tumors, as well as for MAS (43), led to the screening of other G-protein subunits as potentially involved in pituitary oncogenesis. Indeed, all G proteins bind and hydrolyze GTP and share highly conserved primary structures in regions corresponding to the Arg 201 and Gln 227 GNAS mutations. Studies to date, however, failed to confirm the initially promising identification of inactivating mutations in the α-subunit of GIP2, a protein coupled to the inhibitory G-protein Gi2α, whereas no mutations have been identified in the stimulatory Goq or the highly similar Go11 in pituitary and other endocrine tumors (44–51).
Despite the absence of mutations in these genes, overactivity of the protein kinase C (PKC) pathway that is regulated by the Goq and Go11 genes has been suggested in sporadic pituitary adenomas, and the PKCo isoform is found at high levels in a subset of aggressive pituitary tumors (52); some of these tumors are found to contain a somatic mutation (Gly294Asp) at the calcium-binding site (53).

Additional evidence for GNAS involvement in pituitary tumorigenesis comes from recent studies suggesting imprinting of this gene and its 20q13 region in sporadic pituitary adenomas (54). The GNAS gene is transcribed mainly from the maternal allele in almost all endocrine tissues (55); accordingly, GNAS mutations in sporadic pituitary tumors are on the maternal allele and partial loss of this imprinting is present in tumors without GNAS mutations (54).

These data lead to the hypothesis that at least for pituitary somatotrophs, cAMP mediates a mitogenic signal: abnormally high cAMP generation leads to pituitary adenomas. This model is supported by the finding of germline PRKAR1A mutations in CNC, a condition that as we stated above is mostly associated with GH- and PRL-producing pituitary tumors and somatomammotropic hyperplasia. cAMP acts mainly through PKA, a heterotetrameric enzyme that is omnipresent in eukaryotic cells; adequate levels and normal function of PRKAR1A are critical for restraining PKA-dependent serine-threonine phosphorylation of cAMP target molecules: PRKAR1A (and the other PKA regulatory subunits) binds the PKA catalytic subunit when not activated by cAMP (56).

So far 17 of our CNC patients and acromegaly have had mutations of the PRKAR1A gene that result in premature stop codons for the predicted protein sequence; another five patients have been found to have PRKAR1A mutations that lead to an expressed (but abnormal) protein. We could not identify PRKAR1A mutations in three patients with acromegaly and CNC (2). There have been no other gene studies of pituitary tissue from patients with CNC that do not carry germline PRKAR1A mutations. CGH analysis of three CNC-associated micro-adenomas showed no significant changes over normal DNA (29). However, analysis of the most aggressive tumor, an invasive macroadenoma, showed multiple changes, including losses of chromosomal regions 6q, 7q, 11p, 11q, and gains of 1pter-p32, 2q35-qter, 9q33-qter, 12q24-qter, 16, 17, 19p, 20p, 20q, 22p, 22q (29). The greatest contiguous changes were losses of the long arm of chromosome 6 and the entire chromosome 11.

To this date, PRKAR1A mutations have not been found in sporadic pituitary tumors (57–59); likewise, no mutations of any other PKA subunits have been found in sporadic endocrine tumors (60). There has been one ACTH-producing tumor that harbored a GNAS mutation (61), but this observation has not been confirmed in other such cases or in other types of pituitary tumors.

Cell cycle control and related genetic abnormalities in sporadic pituitary tumors

The retinoblastoma gene (RB1) is a tumor-suppressor gene in chromosomal region 13q14.2 that has been linked to pituitary tumors in mice. Losses in this chromosomal region are related to aggressive human pituitary tumor behavior and lack of expression of the protein (pRB) was observed in one fourth of GH-secreting pituitary adenomas (62). However, somatic mutations of the RB1 gene (or the CDK4 gene) do not appear to be present in sporadic pituitary tumors (63). Unless one looks at aggressive tumors, 13q14.2 losses are not frequent in common human pituitary adenomas (64). On the other hand, methylation of CpG islands within the RB1 promoter region was detected in 6 of 10 tumors that failed to express pRb; in addition, one or more exons of the coding region for the protein-binding pocket domain were shown to be homozygously deleted in three of four tumors available for analysis showing that in addition to methylation of the RB1 promoter region, deletions result in loss of detectable pRb expression (65). Thus, it appears that inactivation of RB1 is critical in human pituitary tumor growth and/or expansion, but not for initial tumor formation. RB1 inactivation and p16(INK4a) methylation tend to be mutually exclusive but occasionally coexist with p15(INK4b) methylation. (66).

An elegant experiment that assists in the understanding of the role that loss of RB1 appears to play in pituitary tumor development was that by Loffler et al. (67): the investigators intercrossed mice with targeted deletions of Men1 and Rb1 and compared tumor development in cohorts of animals carrying single or dual mutations of these tumor-suppressor genes. The tumor spectrum in compound heterozygotes was a combination of pathologies seen in each of the individual parental strains without a decrease in the age of onset, indicating independent, non-additive effects of the two mutations, suggesting that menin and RB1 function in a common pathway of tumor suppression (67). Other genes can modify these effects: loss of one or two Nras alleles is shown to significantly reduce the severity of pituitary tumors arising in Rb1−/− animals by enhancing their differentiation (68). Although not studied with relevant mouse models, another possible such genes—‘modifiers’ are GADD45G and MEG3a; the former negatively regulates cell growth and is significantly underexpressed in GH-secreting or PRL-secreting pituitary tumors (69) due to methylation of its promoter CpG island (70); the latter is also underexpressed in human pituitary tumors due to hypermethylation of its promoter (71).

Growth factors and their receptors in pituitary tumors

FGF-4 sequences are present in transforming DNA from human PRL-secreting tumors (72), and FGF-4 facilitates lactotroph proliferation. Changes in cell adhesion have been also correlated with the abnormal expression of pituitary tumor derived FGFR-4 (ptd-FGFR-4) in cell lines and human pituitary adenomas; these changes in expression result in loss of affinity for extracellular matrix (73), which is caused by disruption of a multiprotein complex involving FGFR-4, N-cadherin and neural cell adhesion molecule 1 (NCAM1) (Fig. 1). Expression of ptd-FGFR-4 results is diminished and ectopic cytoplasmic expression of N-cadherin, associated with invasive growth (73). The truncated, kinase-containing variant of FGFR-4 with the alternative
initiation site was isolated from human pituitary tumors (74,75); when expressed in transgenic mice, it led to the formation of invasive pituitary tumors (76).

The epidermal growth factor (EGF) family and its receptors have been implicated in tumorigenesis in a number of neoplasms. Overexpression of TGF-α, under the control of the PRL promoter, has been shown to lead in lactotroph adenomas in transgenic mice (77). In addition, EGFR expression correlates with pituitary tumor aggressiveness, mainly for GH-producing tumors (78).

<table>
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<tr>
<th>Gene</th>
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<th>Human pituitary</th>
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<tr>
<td>TGF-α</td>
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<td>Gsa</td>
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<td>Cyclin E</td>
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<td>Cyclin D1</td>
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<td>Retinoblastoma</td>
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<td>p16</td>
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<td>GADD45γ</td>
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<td>MEG3a</td>
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TGF-α, tumor growth factor α; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GHRH, growth hormone releasing factor; Gsa, stimulatory G protein; PTTG, pituitary tumour transforming gene; GADD45γ, growth arrest and DNA-damage-inducible; MEG3a, maternally expressed gene 3a; GH, Growth Hormone; NFPAs, nonfunctioning pituitary adenomas; TSH, thyroid stimulating hormones; ACTH, adrenocorticotrophic hormone.
PTTG and other genes in human pituitary tumors

The pituitary tumor transforming gene (PTTG) was originally isolated from rat GH and PRL-secreting cells (79,80); it was characterized as an oncogene since it induced neoplastic transformation of NIH 3T3 cells. However, this molecule also belongs to the securin family of proteins that control sister chromatide separation during mitosis (81,82). Thus, alterations in its expression have been proposed as the mechanism underlying the frequent finding of aneuploidy in human pituitary tumors (79), although direct evidence for this is lacking. Currently, three different genes have been identified in the PTTG family, PTTG1, -2 and -3 (83). One of the functions of PTTG in facilitating pituitary tumor development appears to be increased angiogenesis (84). Indeed, like in other neoplasms, angiogenic factors are likely to play a role in pituitary tumorigenesis. These include not only the already mentioned TGF-β but also bFGF, VEGF-A, VEGFR-I and fetal liver kinase 1 (Flk-1), which appear to be associated with an aggressive pituitary phenotype (84–89).

CONCLUDING REMARKS

Genomic approaches have recently been applied to the study of human pituitary tumor biology (90–93); more studies are forthcoming as the technology allows small amounts of tissue to be studied. However, so far, and from the studies that we have so far reviewed in inherited pituitary neoplasias and sporadic tumors, a list of genes (Table 1) and possible pathways to pituitary tumorigenesis emerge (Fig. 2): aberrant...
cAMP signaling is a primary initiating event for hyperplasia and/or adenoma formation, as evidenced by GNAS and PRKARIA (and possibly AIP) involvement; growth of a pituitary tumor is initiated and/or assisted by menin downregulation, methylation of certain target genes, aneuploidy and/or cell cycle disregulation, growth factor and PTTG overexpression, and increased angiogenesis.

Conflict of Interest statement. None declared.

REFERENCES


