New pituitary oncogenes

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Abstract

Pituitary tumors are common monoclonal neoplasms which cause considerable morbidity and mortality. Several molecular events underlying pituitary tumorigenesis have been elucidated in recent years, but no tumor marker has clearly emerged which assists clinical and therapeutic decisions. Activating mutations and loss of inactivating mutations, together with hypothalamic hormones, circulating hormones, growth factors and cytokines cooperatively ensure the inexorable expansion of the initial mutated pituitary cell clone. This review describes new developments in our understanding of the molecular mechanisms involved in the pathogenesis of pituitary tumors. The availability of molecular probes will allow the early prediction of tumor behavior, identify targets for designing subcellular pituitary tumor therapy and provide novel approaches to pituitary tumor management.

Introduction

Detailed histological studies in unselected autopsies have identified pituitary tumors in up to 27% of pituitary tissues (Costello 1936, Burrow et al. 1981), and high resolution computed tomography and magnetic resonance imaging performed on a general population show incidental clinically silent pituitary tumors measuring 3 mm or more in diameter in approximately 20% of apparently ‘normal’ pituitary glands (Kovacs & Horvath 1986). Pituitary tumors account for 10–15% of all intracranial neoplasms (Terada et al. 1995), are infrequent in childhood (Mukai et al. 1986, Kane et al. 1994) and are more common in females (Kane et al. 1994). Women usually present at a younger age, most commonly with prolactin (PRL)- and adrenocorticotropin (ACTH)-secreting tumors. The most common presentation is with menstrual or fertility dysregularity secondary to hyperprolactinemia. ACTH excess causing Cushing’s or Nelson’s syndromes or growth hormone (GH) hypersecretion leading to acromegaly are other syndromes which may present to the physician. Thyrotropin (TSH)-secreting hormone-producing tumors, and gonadotroph (follicle stimulating-hormone (FSH) and/or luteinizing hormone (LH) secreting) adenomas are comparatively rare and the remaining endocrinologically silent tumors grow insidiously for many years. Men tend to present in middle age with clinically non-functioning tumors which lead to compressive hypopituitarism, visual deficit, or headache secondary to an expanding sellar mass (Mukai et al. 1986). Pituitary tumors rarely metastasize outside the pituitary bed but large invasive tumors are commonly encountered, and microscopic dural invasion occurs frequently. Therefore despite their ‘benign’ categorization, these tumors cause considerable post-operative patient morbidity and ultimately mortality, and often present diagnostic and management challenges.

Our understanding of many of the molecular events involved in pituitary tumor pathogenesis has increased considerably, but current tumor markers cannot accurately identify those patients who require intensive initial therapy for their pituitary tumor and close follow-up. This review describes several new developments in our understanding of the molecular mechanisms involved in the pathogenesis of pituitary tumors.

Intrinsic pituitary lesion or hypotalamic origin

Highly differentiated mature cell types populate the anterior pituitary and each of these may give rise to unique tumor types. Commitment of pituitary cell function is governed by a variety of cell-specific transcriptional factors which determine the final pituitary phenotype. Expression of these cell-specific pituitary genes is closely regulated by hypothalamic
and peripheral hormones as well as paracrine growth factors. Virtually all functional and non-functional pituitary tumors are monoclonal in origin, indicating that an intrinsic defect in a single pituitary cell is the primary event in pituitary tumor pathogenesis (Alexander et al. 1990, Herman et al. 1990, Melmed 1999) (Fig. 1). However, it is likely that hypothalamic hormones, local growth factors and circulating sex steroid hormones are also implicated in pituitary tumor pathogenesis by creating a permissive environment which potentiates cell mutation and subsequent clonal expansion of the initial mutated cell. In some surgical series, detection of more than one tumor has been reported, and in random autopsies 0.9% of pituitary adenomas were of multiple origin (Kontogeorgos et al. 1991). However, adenohypophyseal tissue surrounding pituitary tumors is normal (Molitch & Russell 1990), supporting the notion that multiple independent cellular events such as generalized hyperplasia do not necessarily precede adenoma formation.

**Candidate genes in human pituitary tumors**

During pituitary cellular transformation, a spectrum of successive genetic alterations are observed, which include dysregulation of cell proliferation, differentiation, and specific hormone production. This multistep transformation process encompasses seemingly different molecular events leading to similar tumor phenotypes and it is the accumulation of alterational events rather than the temporal order in which they occur which appears to be important (Fearon & Vogelstein 1990). Furthermore, the transformation process quickly gains momentum as each new ‘hit’ renders the

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**Figure 1** Model of pituitary tumorigenesis. Cells responding to endocrine or paracrine stimuli may expand in a polyclonal manner. This ‘non-committed’ cell may return to a normal pituiticyte, undergo apoptosis, or acquire activating mutations or loss of inactivating mutations, prompting the emergence of a monoclonal cell population (light shaded arrow). Alternatively, a normal cell may acquire sufficient activating mutations or loss of inactivating events to prompt a rapidly expanding monoclonal cell population from the onset (lower sequence). Following additional genetic events, this monoclonal expansion may evolve into an invasive pituitary tumor, with further events promoting the progression to metastatic pituitary carcinoma. The progress of both these pathways will be driven by a variety of hormonal stimuli, growth and angiogenic factors, and altered receptor expression (dark shading). ER = estrogen receptor.
cell susceptible to subsequent mutational events. Numerous extrinsic factors and intrinsic pituitary defects may cause irreversible pituitary cell DNA mutations (Table 1) leading to a broad spectrum of molecular abnormalities.

Two categories of genes promote the outgrowth and evolution of a neoplastic clone. Oncogenes bear a ‘gain-of-function’ mutation in their regulatory or coding regions, leading to either the formation of an abnormal protein product or dysregulation of their normal product. The proto-oncogene, which is the normal non-mutated endogenous version of an oncogene, can be activated by chromosomal translocations or inversions, point mutations or fusion of key components of its coding region with another gene.

Tumor suppressor genes normally act to restrain cell proliferation, by regulating the cell cycle or by maintaining genomic stability, thus preventing other malignant cell manifestations. Gene deletions or point mutations lead to ‘loss of function’ of both alleles of a tumor suppressor gene. The ensuing inactivation or elimination of the functional protein product leads to tumor formation.

Tumor suppressor genes

Loss of heterozygosity (LOH) involving chromosomes 11q13, 13 and 9 occurs in up to 20% of sporadic pituitary tumors (Herman et al. 1993, Boggild et al. 1994, Farrell et al. 1997), mainly in macroadenomas or invasive tumors. Although allelic loss of a retinoblastoma RB1 intragenic marker on chromosome 13q has been described in aggressive and metastatic pituitary tumors (Woloschak et al. 1992, Pei et al. 1995, Simpson et al. 1999), only a single study has observed associated loss of Rb protein in these tumors (Simpson et al. 1999), suggesting that an alternate tumor suppressor gene in this region may be involved in controlling the propensity for tumor invasion.

Multiple endocrine neoplasia type 1 (MEN-1) gene

MEN is an autosomal dominant disorder, features of which include pituitary and parathyroid adenomas, and/or tumors of the endocrine pancreas. The MENIN tumor suppressor gene maps to chromosome 11q13 (Larsson et al. 1998, Qian et al. 1998), and encodes a 610 amino acid protein nuclear product (Chandrasekharappak et al. 1997, Guru et al. 1998). However, despite LOH in the 11q13 region, MENIN RNA and menin protein product are appropriately expressed in sporadic pituitary adenomas (Prezant et al. 1998, Tanaka et al. 1999) and, similar to 13q LOH, another candidate gene in this region is now being sought.

Cyclin-dependent kinase (CDKI) inhibitors

In mammalian cells, CDK4 and CDK6 enzymes in combination with three D-type cyclins (D1, D2 and D3), and CDK2 in association with cyclin E, play distinct roles in regulating G1 progression. CDK4/6–cyclin D couple extracellular growth signals to cell cycle substrates, such as the Rb protein, while CDK2–cyclin E controls initiation of DNA replication (Sherr & Roberts 1995). The activity of these cyclin–CDK complexes is regulated by the INK4 (p16, p18, p19) and Kip/Cip (p21, p27, p57) families of CDKI proteins (Weinberg 1995). Frequent LOH of 9p21, the locus of the CDKN2A gene, has been reported in pituitary adenomas and low expression of its protein product, p16, has been described in pituitary tumor tissue extracts. Interestingly, mice lacking p16, while developing spontaneous tumors at multiple sites and at an early age, do not display a pituitary abnormality (Serrano et al. 1996). Another member of the INK4 family, p18 protein, functions similarly to p16, inactivating CDK and hence inhibiting Rb phosphorylation.

Targeted disruption of either p18INK4 or p27kip1 in transgenic mice produced animals with increased body size, multiorgan hyperplasia, female sterility and pituitary tumors (Franklin et al. 1999). Mice lacking both p18 and p27 died from pituitary adenomas at 3 months, suggesting that p18 and p27 mediate two separate pathways which synergistically suppress pituitary tumorigenesis (Franklin et al. 1999). Although p27 mRNA levels are normal in human pituitary tumors, a recent study has demonstrated low p27 protein expression in pituitary tumors in comparison with normal pituitary tissue (Lidhar et al. 1999).

Tumor suppressor gene abnormalities have been observed mostly in large invasive pituitary tumors, appear to be relatively late events and as yet, no clear-cut loss of tumor...
suppressor activity which results in increased pituitary proliferative activity has emerged. Therefore, the specificity of these LOH patterns is unclear and they imply that earlier genetic abnormalities predispose cells to the LOH patterns, which accelerate subsequent cell growth.

Oncogenes

Stimulatory guanine nucleotide-binding protein (G-protein) α-subunit gene (gsp)
The stimulatory G (Gs) protein activates adenyl cyclase and somatotroph cAMP accumulation. Point mutations of residue 201 (Arg → Cys or His) or 227 (Gln → Arg or Leu) result in ligand-independent constitutive activation of cAMP and GH hypersecretion. The missense mutations (Gsp) have been demonstrated in ~40% of GH-secreting pituitary tumors in Caucasians and a lower incidence reported in Japanese subjects (Yoshimoto et al. 1993) with acromegaly, possibly reflecting a geographical or ethnic influence. Although the presence of Gsp mutations in GH-secreting pituitary tumors is readily apparent, their clinical significance is not apparent and Gsp mutations do not provide a unifying mechanism of oncogenesis in pituitary tumors as these mutations are infrequently observed in other pituitary tumor subtypes (Spada et al. 1990).

Activated cAMP-response element binding proteins (CREB)
cAMP may stimulate somatotroph GH transcription directly mediated by the cAMP-responsive nuclear transcription factor (CREB). Transgenic mice overexpressing a phosphorylation-deficient and transcriptionally inactive mutant of CREB in the anterior pituitary exhibit dwarfism and somatotroph hypoplasia (Struthers et al. 1991), indicating that phosphorylated CREB plays a role as a biochemical intermediate in the somatotroph proliferative response. Significantly higher amounts of Ser152-phosphorylated, and hence activated, CREB were detected in a series of GH-secreting pituitary tumors compared with a group of non-functioning tumors. Therefore, constitutively activated CREB, possibly promoted by Gs-α overexpression, may facilitate somatotroph transformation (Berthrat et al. 1995).

Ras

This family of three related ras proto-oncogenes (H-ras, K-ras and N-ras), encode 21 kDa proteins which are structurally similar to the membrane anchored G-proteins. H-ras mutations have been identified in metastases from pituitary carcinomas (Cai et al. 1994, Pei et al. 1994) and in a single aggressive PRL-secreting pituitary adenoma (Karga et al. 1992). ras activation in pituitary tumors is thus a late sequela, unlike other human tumors, such as colorectal or thyroid cancer, where ras activation is considered an early event.

Pituitary tumor transforming gene (PTTG)

Recently we isolated a novel pituitary tumor transforming gene (PTTG) by differential display PCR using mRNA derived from rat pituitary tumor cells (GH4) and normal pituitary tissue (Pei & Melmed 1997). Subsequently we cloned the human homologue of this transforming gene from a fetal liver cDNA library. The nucleotide sequence of human PTTG (hPTTG) cDNA is 85% identical to rat PTTG cDNA and the encoded proteins are 89% similar (Zhang et al. 1999a). The 609 bp open reading frame of hPTTG encodes a unique protein which may be the index member of a novel family of human securin-like proteins. PTTG associates with another nuclear separin protein, Esp1p, to inhibit chromatid separation during mitosis (Zou et al. 1999). Increased expression of PTTG/securin would result in disrupted chromatid separation, resulting in chromosomal gain or loss. The subsequent chromosomal aneuploidy and genetic instability may lead to activation of proto-oncogenes or LOH of tumor suppressors. The human PTTG family consists of at least three homologous genes, of which PTTG1 is located on chromosome 5q33 (Prezant et al. 1999). Weak PTTG expression is observed in most normal adult tissues including colon, small intestine, brain and pancreas with stronger PTTG expression found in thymus, and abundant expression in testis. In contrast, abundant PTTG expression is observed in all cancer cell lines tested. PTTG overexpression has also been reported in several solid human tumors including pituitary, ovary, endometrium, liver, adrenal and kidney and also in a variety of hematopoietic neoplasms (Domínguez et al. 1999, Zhang et al. 1999b).

Transfectants overexpressing PTTG cDNA were tested for transformation as assayed by anchorage-independent growth in soft agar. NIH3T3 fibroblasts overexpressing PTTG formed large colonies on soft agar in comparison with minimal colony formation with mock-transfected control cells, and when PTTG-transfected cells were injected into athymic nude mice, large tumors formed within 2 weeks in all animals (Pei & Melmed 1997, Zhang et al. 1999a). The PTTG protein contains a proline-rich (P-X-X-P) potential SH-3 docking motif, suggesting that it may be involved in intracellular signaling. In addition to its role in cellular transformation, conditioned medium from wild-type PTTG-transfected cells contained higher levels of basic fibroblast growth factor (bFGF) and these cells expressed higher bFGF mRNA levels that controlled transfected cells. Thus, it appears that PTTG stimulates bFGF expression and secretion (Zhang et al. 1999a). Mutations of the proline-rich region of the PTTG protein prevents the ability of PTTG to cause in vitro transformation,
in vivo tumorigenesis and bFGF induction, indicating the importance of this SH3-binding motif. bFGF is a potent angiogenic factor which is expressed in both normal pituitary tissue and pituitary tumors and also stimulates PRL secretion in vitro (Larson et al. 1990). Patients with MEN-1 harboring pituitary adenomas had elevated serum immunoreactive bFGF levels and these were lowered significantly following medical or surgical treatment (Zimmering et al. 1993).

**PTTG in pituitary tumors**

In the normal human pituitary, even using sensitive methods such as RT-PCR, PTTG expression is low. In contrast, more than 50% increases in PTTG expression were observed in 23 out of 30 non-functioning pituitary tumors, all 13 GH-secreting tumors, 9 out of 10 prolactinomas and the single ACTH-secreting tumor examined, with more than 10-fold increases in PTTG expression evident in some tumors (Zhang et al. 1999b). Furthermore, highest PTTG expression was observed in hormone-secreting pituitary tumors which had invaded the sphenoid bone (Stages III and IV; 95% confidence interval (CI), 3.1- to 9.7-fold increase) compared with tumors confined to the pituitary fossa (Stage I and II; 95% CI, 1.7- to 3.0-fold PTTG increase) compared with tumors confined to the pituitary fossa (Stage I and II; 95% CI, 1.7- to 3.0-fold PTTG increase), and concordant bFGF and PTTG mRNA expression was observed in all pituitary tumors (Fig. 2) (Heaney et al. 1999). Preliminary in situ hybridization studies demonstrate that PTTG expression is localized in pituitary adenoma cell cytoplasm in contrast to the normal pituitary in which no PTTG expression was observed. PTTG is therefore the first human transforming gene found to be overexpressed in the majority of pituitary tumors tested and may therefore be a novel marker of invasiveness in secreting pituitary tumors.

In addition to increased expression in human pituitary tumors, we also examined the pattern of PTTG expression during early experimental pituitary cell transformation. Highest PTTG expression was observed in prolactinomas, and we therefore chose to generate lactotroph tumors by administration of estrogen to F344 rats. Following estrogen administration for 4 weeks, rats developed large pituitary tumors (300% increase in size) along with an expected increase in pituitary PRL mRNA expression and serum PRL level. In addition, examination of pituitary tissue extracts from these animals revealed a ~6-fold induction of pituitary pttg mRNA, and estrogen-induced pituitary pttg in vivo was observed to increase in a time- and dose-dependent manner (Fig. 3) (Heaney et al. 1999).

As well as increased expression of pituitary pttg, we observed pituitary histological changes. Twenty-four hours after commencement of estrogen, when pituitary pttg was first induced, hypertrophy of PRL-secreting cells was visible. Maximal induction of pituitary pttg was seen after 48 h estrogen treatment, at which time groups of PRL-immunopositive cells close to newly formed blood vessels were observed. Displacement of the reticulin fibers around the lactotroph cells, a feature typical of lactotroph hyperplasia, was apparent 1 week after commencement of the estrogen infusion. Complete disruption of reticulin fibers, in association with vacuolation and nuclear pleomorphism heralded the appearance of true adenoma formation and was apparent 4 weeks after commencement of estrogen. These results indicate that estrogen-induced pttg expression is coincident with early pituitary lactotroph transformation (normal cell → hypertropic/hyperplastic cell), and the appearance of newly formed pituitary arterial networks.

In view of our previous findings that pttg regulated bFGF secretion and the close correlation of PTTG and bFGF expression in human pituitary tumors, we also examined bFGF expression in experimental rat lactotroph tumors. Western blot and immunocytochemical analysis showed that increased pttg and bFGF expression throughout the pituitary occurred 24 h after commencement of estrogen infusion and was most prominent 48 h after estrogen commencement. We also examined immunoreactivity to vascular endothelial growth factor (VEGF) in the experimental rat tumors. Increased VEGF immunoreactivity was seen 24 h after estrogen administration, and was visible throughout the pituitary. Immunoreactivity to both bFGF and VEGF immunoreactivity were particularly prominent at the edges of PRL-secreting tumors which invaded the pituitary capsule (Heaney et al. 1999). Our findings that PTTG, bFGF and VEGF expression occur early (24 h) and co-incidentally in estrogen-induced pituitary lactotroph transformation, and that higher bFGF expression is seen at invasive tumor margins provide strong evidence in favor of a novel growth factor-mediated mechanism for pituitary cell transformation and pituitary tumor progression and invasion.

**Estrogen and bFGF regulate pttg in vitro**

In view of our observations in experimental rat lactotroph tumors, and in order to further study the regulation of pituitary pttg by estrogen, we then examined effects of estrogen administration on the regulation of rat pttg and the relationship between bFGF and pttg in vitro. Rat pituitary GH/PRL-secreting cells (GH3) were incubated in medium containing serum which had been pretreated with activated charcoal (CSS) to reduce steroid concentrations. Treatment of the steroid-depleted GH3 cells with diethylstilbestrol (10^{-5}–10^{-8} M), produced a ~6-fold increase in pttg mRNA expression (P < 0.01) and this increase was first observed 12 h after the addition of diethylstilbestrol and was maximal at 24 h (P < 0.05). Highest pttg expression was observed in GH3 cells which had been incubated in whole serum (WS)-supplemented medium. This estrogen- or WS-mediated pttg induction was markedly inhibited by co-incubation of GH3 cells in
Figure 2 (Upper panel) Representative RT-PCR analysis of PTTG1 and bFGF expression. Left margin, molecular size; right margin, positions of RT-PCR products. NP = normal pituitary. (Lower panel) Graphic representation (Pearson correlation) of PTTG1 and bFGF mRNA densitometry values. Forty-one secretory and non-secretory human pituitary tumors and two normal human pituitary tissues were analyzed. NF = non-functioning (n = 15); GH = GH-secreting (n = 8); PRL = PRL-secreting (n = 7); ACTH = ACTH-secreting (n = 1); TSH = TSH-secreting (n = 1); mixed, plurihormonal immunoreactivity (n = 8). (From Heaney et al. 1999, with permission).

CSS-treated medium containing diethylstilbestrol or WS and 1000- (diethylstilbestrol, P < 0.01; WS, P < 0.05) or 100-fold (diethylstilbestrol/WS, P < 0.05) excess of the anti-estrogen, ICI-182780 (Heaney et al. 1999). Treatment of GH3 cells with tri-iodothyronine (5 nM) led to increased expression of PRL mRNA but did not significantly alter pttg induction. To examine the mechanism of the estrogen-mediated pttg mRNA induction in more detail, we then transfected the full-length mouse pttg promoter-luciferase reporter construct (Wang & Melmed 2000) (4.2 kb) into the GH3 cells. A ~220% increase (P = 0.02) in pttg-luciferase activity was seen in the pttg-transfected GH3 cells following treatment with diethylstilbestrol (10^{-10} M) and this estrogen-induced pttg-promoter activity was partially inhibited by co-incubation of the transfected GH3 cells with estrogen and anti-estrogen (ICI-182780, 10^{-7} M) (P = 0.02). The murine pttg promoter contains several putative estrogen response elements, which provide a potential mechanism for our observed estrogen-induction of pttg. We had previously observed that overexpression of PTTG in 3T3 fibroblasts led to increased bFGF expression and secretion. We sought to further elucidate a paracrine mechanism for pituitary PTTG regulation, and as NIH3T3 cells, unlike the GH3 cells, constitutively exhibit low PTTG expression, we treated these cells with bFGF (1 ng/ml). After 24 h of bFGF treatment, a ~240% induction of pttg mRNA was observed, which was blocked by co-incubation with a neutralizing bFGF antibody. Administration of bFGF for intervals less than 24 h did not induce pttg. As pttg stimulates bFGF secretion and bFGF also induces pttg, these studies suggest the existence of an autocrine/paracrine regulatory loop in pituitary pttg regulation.
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Figure 3 In vivo estrogen induction of PTTG in rat lactotroph tumors. Representative rat pituitary tumor, serum PRL and pituitary wet weight (upper panel) and Northern (middle panel) and Western blot (lower panel) of pituitary tissue extracts derived from estrogen-treated rats (filled bars), intact male rats (n = 2) and ovariectomized controls (Ovx, n = 2, clear bars) during prolonged estrogen treatment. Molecular size standards (M), pttg, β-actin (internal control) and bFGF mRNA (middle panel) and PTTG protein induction (lower panel). (From Heaney et al. 1999, with permission).

Growth factors, cytokines, steroid hormones and their receptors in pituitary tumorigenesis: permissive and/or causative roles

A generalized proliferative polyclonal cell expansion may be physiological, such as the pituitary lactotroph hyperplasia observed during pregnancy. Distinct from true neoplasms which are monoclonal growths, a generalized stimulus to polyclonal hyperplasia in the pituitary, perhaps growth factor- or hormonally mediated can foster the emergence of a monoclonal cell population, by increasing the odds of mitosis-related cellular DNA damage. Several polypeptide growth factor families may promote pituitary tumorigenesis: some oncogene products share homology with growth factors or their receptors; autocrine or paracrine-growth factor receptor interactions regulate cell growth and gene expression; and growth factors modulate hormone production. The majority of the growth factors identified in the pituitary play a role in supporting development and expansion of an established monoclonal growth, but few have been convincingly shown to cause initial cellular transformation. For example, targeted overexpression of bFGF in transgenic mice leads to lactotroph hyperplasia, but does not progress to true adenoma formation. In contrast, transgenic mice overexpressing transforming growth factor (TGF)-α under the control of the PRL promoter in mice, develop lactotroph adenomas (McAndrew et al. 1995).

FGF-4

FGF-4, the protein product of the heparin-binding secretory transforming gene (hst) gene (Sakamoto et al. 1986), is expressed in PRL-secreting pituitary adenomas and possesses potent angiogenic activity, and experimental overexpression of transected hst oncogene enhances PRL secretion and is associated with tumor cell aggressiveness (Shimon et al. 1996). FGF-4 immunoreactivity is observed in 33% of PRL-secreting pituitary adenomas as compared with absent immunoreactivity in normal pituitary tissue and other adenoma subtypes (Shimon et al. 1998). However, no hst gene rearrangement has been detected in human prolactinomas, and the mechanism by which hst/FGF-4 complex initiates or promotes lactotroph proliferation and PRL secretion is unclear.

Other growth factors and cytokines

Interleukin-6 (IL-6), IL-8, IL-11, and leukemia inhibitory factor (ILF) overexpression have been demonstrated in pituitary adenomas (Auernhammer & Melmed 1999, Renner et al. 1998, Sulliman et al. 1999), but as folliculostellate cells produce IL-6, and most studies have employed RT-PCR of whole pituitary extracts, it is unclear if tumor cells themselves produce and/or are responsive to ILs, thus potentially affording a role for ILs in pituitary tumor pathogenesis.

Estrogen and estrogen receptor

Estrogen is a powerful stimulator of cellular proliferation in the pituitary, as evidenced by the diffuse hyperplastic proliferation of the PRL-secreting cells which occurs during pregnancy or lactation (Kovacs & Horvath 1986). At term, immunopositivity to PRL is observed in 60–70% of adenohypophyseal cells, and though these changes mostly revert to
the prepregnant state, multiparous women demonstrate higher pituitary weight and PRL cell number than nulliparous women (Asa et al. 1982). Unopposed estrogen administration has been implicated in the pathogenesis of prolactinoma in several male–female transsexual individuals (Kovacs et al. 1994).

Cellular receptor expression in addition to cellular environmental ligand concentrations combine to determine pituitary cell responsivity to specific factors, including estrogen. Using a variety of techniques, all pituitary tumor types have been shown to express estrogen receptors, suggesting a role for estrogen in pituitary tumor growth (Stefeananu et al. 1994). Highest estrogen receptor expression is observed in prolactinomas, which are the most ‘estrogen-responsive’ pituitary tumors (Nakao et al. 1989, Jaffrain-Rea et al. 1996). Significant estrogen receptor expression has also been described in mixed somatotroph (GH/PRL) and gonadotroph (FSH and/or LH) pituitary tumors. In contrast, non-functioning pituitary tumors, which do not exhibit gonadotroph immunoreactivity, have lowest estrogen receptor expression (Jaffrain-Rea et al. 1996). Furthermore, although males have lower circulating estradiol levels, higher estrogen receptor expression has been described in PRL-secreting pituitary tumors derived from males in comparison with PRL-secreting pituitary tumors from females and macro-adenomas (size ≥1 cm) of all types exhibit higher estrogen receptor expression than in comparison with microadenomas (size <1 cm) (Nakao et al. 1989, Stefeananu et al. 1994). The higher estrogen receptor expression may partly explain why macroprolactinomas in males tend to be more invasive.

**Transcription factors**

The process of adenohypophyseal differentiation is a highly specific and temporally regulated series of events (Barlier et al. 1999). An increasing number of putative transcription regulating factors are key factors for determination of cell specificity in the pituitary and the regulation of hormone gene expression. PTx-1, pituitary homeobox factor, an activator of proopiomelanocortin (POMC) gene expression (Lamonerie et al. 1996) plays a role in brain and facial development and PTx-1 expression has been demonstrated in all normal anterior pituitary cell types and the majority of all pituitary adenoma subtypes (Barlier et al. 1999).

A single study has described absent Ptx-2 expression in corticotroph adenomas, high Ptx-2 expression in gonadotroph tumors, and although pure lactotroph tumors expressed Ptx-2, no expression was observed in somatotroph adenomas. This suggests a role for Ptx-2 in the terminal differentiation of the somatotroph and lactotroph cell phenotype. Although inactivating mutations of the prophet of Pit-1 (PROP-1), so-called as it is necessary for Pit-1 gene expression, have been found in human subjects with combined pituitary hormone deficiency (Wu et al. 1998) and in Ames dwarf mice (Sornson et al. 1996), RT-PCR analyses have demonstrated Prop-1 expression in normal pituitary tissue and all pituitary adenoma subtypes (Nakamura et al. 1999). Therefore, no clear role has yet emerged for these transcription factors in pituitary tumor pathogenesis as their ubiquitous pituitary expression appears independent of hormonal regulation and tumor phenotype.

**Angiogenesis**

Angiogenesis is a discrete component of the tumor phenotype, is a potential rate-limiting step in the developmental pathway of solid tumors (Folkman 1992), and is modulated by a variety of cytokines and growth factors by a paracrine mode of action (Godspodarowicz et al. 1987, Bikfalvi et al. 1997, Ferrara & Davis-Smyth 1997). The potent angiogenic factor, bFGF, is localized in the basement membranes and extracellular matrix of pituitary endocrine and folliculostellate cells. In vitro, bFGF regulates rat GH, PRL and TSH secretion (Baird et al. 1985), and bFGF derived from normal pituitary tissue and pituitary tumors stimulates PRL secretion (Schechter & Weiner 1991). Furthermore, patients with MEN-1 harboring pituitary adenomas were found to have elevated serum immunoreactive bFGF levels and these fell significantly following medical or surgical treatment (Zimmering et al. 1993).

In the well-characterized estrogen-induced F344 rat pituitary lactotroph tumor model, a striking increase in vascularity occurs, in association with increases in the angiogenic factors, bFGF (Schechter & Weiner 1991) and VEGF (Banerjee et al. 1997). We have demonstrated increased bFGF and VEGF expression early after commencement of estrogen administration and preceding the appearance of mature vessels in the rat anterior pituitary (Heaney et al. 1999). In the human anterior pituitary, angiogenesis has particular significance for PRL cell transformation. In normal circumstances, dopamine released from median eminence nerve terminals tonically inhibits PRL-secreting lactotrophs. Destruction of the dopamine-secreting tuberoinfundibular neurons increases serum PRL levels and lactotroph density (Schechter & Weiner 1991), and dopamine D2 receptor-deficient mice develop lactotroph adenomas (Asa et al. 1999). In a similar manner, subphysiological pituitary dopamine concentrations as a consequence of pituitary–systemic anastomoses would allow lactotrophs to escape dopamine inhibition (Weiner et al. 1985). Indeed, PRL-secreting adenomas are located in the lateral wings of the pituitary gland, have a close relationship with pituitary capillaries (Kovacs et al. 1978) and well-formed dural-derived arteries directly enter the anterior pituitary in 80% of human PRL-secreting pituitary tumors (Schechter et al. 1988). Both the VEGF 165 and VEGF 189 protein isoforms of VEGF have been detected in virtually all pituitary tumors examined to
date (Nishikawa et al. 1998), and pituitary VEGF is positively regulated in vitro by pituitary adenylate cyclase-activating peptide and IL-6 (Pagotto et al. 1999). These preliminary observations emphasize the complex interactions and paracrine/autocrine ‘cross-talk’ which occurs in the pituitary involving hormones, oncogenes, cytokines, growth and angiogenic factors, and contribute to pituitary tumor development and progression.

Hypothalamic factors and their receptors

A variety of hypothalamic- and pituitary-derived polypeptides regulate gene expression and pituitary hormone secretion and may promote pituitary tumor development.

GH-releasing hormone (GHRH) and somatostatin

GHRH induces somatotroph proliferation and DNA synthesis, targeted overexpression of GHRH in transgenic mice produces mammosomatotroph hyperplasia and mammosomatotroph adenomas (Asa et al. 1990), and extra-hypothalamic tumors secreting ectopic GHRH induce somatotroph hyperplasia and acromegaly (Melmed 1998, Sano 1998). Somatotroph adenoma cells are responsive to GHRH in vitro (Adams et al. 1983, Spada et al. 1987) and, although some pituitary tumors express a truncated GHRH receptor (Hashimoto et al. 1995), these mutations are not associated with constitutive GHRH receptor activation.

Pituitary adenomas express somatostatin receptor subtypes (Greenman & Melmed 1994a,b) but in contrast to normal pituitary tissue, where both somatostatin precursors and somatostatin are expressed, mature somatostatin is not detected in GH-secreting adenomas (Levy et al. 1993).

Corticotropic-releasing hormone (CRH)

ACTH-secreting pituitary adenomas express both the CRH and the closely related vasopressin V3 receptor, and they exhibit POMC mRNA expression following calcium stimulation in vitro (Suda et al. 1983). Although Cushing’s disease is due to a pituitary microadenoma in 90% of cases, pituitary corticotroph hyperplasia has been described in association with ectopic CRH production (Asa et al. 1984), long-standing Addison’s disease (Scheithauer et al. 1983), and in animals after continuous CRH infusion (Gertz et al. 1987, McNichol et al. 1988).

TSH-releasing hormone (TRH)

Thyrotroph hyperplasia in patients with long-standing primary hypothyroidism (Scheithauer et al. 1985, Sarlis et al. 1997) is usually reversible with replacement doses of thyroid hormone and is most commonly described in young females (Horvath et al. 1999). Although normal pituitary and some pituitary adenoma cells produce TRH (Le Dafni et al. 1989, 1990), and low β₁- and β₂-isomorph expression has been demonstrated in non-functioning pituitary tumors in comparison with normal pituitary tissue, TRH signaling appears to be intact and no activating mutations have been identified in pituitary tumors (Dong et al. 1996).

Gonadotropin-releasing hormone (GnRH)

Gonadotroph adenomas in patients with long-standing hypogonadism are well documented (Snyder 1985, Riedl & Frisch 1997), but gonadotroph hyperplasia is rare (Nicolis et al. 1988, Okuda et al. 1989, Horvath et al. 1999) and although pituitary adenomas express both GnRH, intact GnRH and truncated GnRH receptors (Alexander & Klibanski 1994), no activating GnRH receptor mutations have thus far been described.

Summary and future directions

Our current knowledge of the mechanisms involved in pituitary tumor pathogenesis show the uniqueness of this tissue which exemplifies the complex interplay between hormones, growth factors, oncogenes and tumor suppressor genes. Further characterization of the many factors, known and as yet undiscovered, and the availability of molecular probes will allow the early prediction of tumor behavior, portend responses to therapeutic interventions, and, in the future, provide screening tests for pituitary tumor prediction. Rational approaches to subcellular therapy for somatotroph adenomas are also now feasible experimentally.

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