Brain Pathology 11: 356-370(2001)

SYMPOSIUM: Pathology and Pathogenesis of Diseases of the Pituitary Gland

The Role of Hormones, Growth Factors and Their Receptors in Pituitary Tumorigenesis

Shereen Ezzat

Department of Medicine, University of Toronto, and The Freeman Centre for Endocrine Oncology, Mount Sinai Hospital, Toronto, Ontario, Canada

This work was supported in part by grants from the Canadian Institutes of Health Research (MT-14404).

Numerous factors have been shown to govern adenohypophysial cell proliferation. Human and animal models have documented that the hypothalamic trophic hormone growth hormone-releasing hormone stimulates cell proliferation, and prolonged stimulation leads to tumor formation. Similarly, lack of dopaminergic inhibition of lactotrophs and lack of feedback suppression by adrenal, gonadal or thyroid hormones are implicated, perhaps through hypothalamic stimulatory mechanisms, in pituitary adenoma formation superimposed on hyperplasia. However, most pituitary tumors are not associated with underlying hyperplasia. Overexpression of growth factors and their receptors, such as EGF, TGFα, EGF-R and VEGF has been identified in pituitary adenomas, and reduction of follistatin expression has been implicated in gonadotroph adenomas. Aberrant expression of members of the FGF family, an FGF antisense gene and FGF receptors have all been described in pituitary adenomas. The clonal composition of pituitary adenomas attests to the molecular basis of pituitary tumorigenesis, however, the evidence suggests that these various hypophysiotropic hormones and growth factors likely play a role as promoters of tumor cell growth in genetically transformed cells.

Introduction

Pituitary tumors are common neoplasms that exhibit a highly variable biological course as evidenced by hormonal and proliferative activities. Progress in defining the factors that govern cell differentiation in the pituitary have led to a new classification of anterior pituitary adenoma tumor cell types (9). Traditionally, there had been an on-going controversy regarding the basis of pituitary tumorigenesis. Two prevailing theories confronted hormonal stimulation against an intrinsic pituitary defect. Several animal models and unusual clinical cases have provided support for the role of hormonal stimulation in the development of these neoplasms and there is evidence for intra-pituitary production of hypothalamic hormones that may be responsible for excess stimulation. In contrast, the clonal nature of pituitary adenomas and the lack of associated hyperplasia accompanying pituitary adenomas strongly argue for an intrinsic somatic defect(s) as the principal etiology contributing to the genesis of these lesions.

Hormonal influences

Evidence suggestive of a possible hormonal etiology in pituitary tumorigenesis includes paradoxical pituitary hormone responses to exogenous peptide stimulation, the development of pituitary adenomas in situations of excessive hypothalamic hormone stimulation or loss of negative feedback inhibition by target gland hormones, and evidence of hypothalamic hormone production within the anterior pituitary.

Persuasive arguments against a hormonal etiology in human pituitary tumorigenesis includes the rarity of hyperplastic changes associated with pituitary adenomas, the lack of true adenomatous changes in the pituitary even after sustained exposure to hypothalamic hormones, and the relatively low frequency of recurrence following successful adenoma resection.

Excess hormonal stimulation. *Growth hormone-releasing hormone (GHRH).* GHRH can cause somatotroph proliferation (25) and somatotroph hyperplasia is well documented as a consequence of chronic stimulation in patients with endocrine carcinomas ectopically secreting GHRH (127, 142). Intra-pituitary GHRH expression is well documented (66, 85, 110) and GHRH may be over-expressed in some aggressive tumors (141). *In vitro*, human somatotroph adenomas respond to GHRH stimulation (4, 5, 71, 90, 136, 150) consistent

Corresponding author:
Shereen Ezzat, University of Toronto, 600 University Ave. Rm 437, Toronto, Ontario, Canada M5G-1X5; Tel.: (416) 586-8505; Fax: (416) 586-8834; E-mail: sezzat@mtsinai.on.ca

with functional GHRH receptors on these tumors. In some instances, however, they may lose the down-regulation characteristic of normal somatotrophs (71, 136). Thus, it would appear that GHRH stimulation may play a contributory role in the development of pituitary adenomas. Moreover, older transgenic mice over-expressing GHRH develop pituitary adenomas (Figure 1) (13, 89). However, in situations of GHRH excess, GH-producing adenomas are usually accompanied by somatotroph hyperplasia which is distinctly unusual in sporadic human pituitary GH adenomas (123). Moreover, sustained exposure to ectopic GHRH does not always lead to true adenoma formation (35).

Cloning of the GHRH receptor has permitted examination of the role of GHRH in somatotroph function. Indeed, loss of GHRH receptor signaling is now recognized as the genetic basis for the little (lit/lit) dwarf mouse (49, 88). A truncated alternatively spliced form of the GHRH receptor with limited signaling properties has been identified in GH-producing pituitary adenomas (58). The GHRH receptor expression does not appear to be restricted to somatotroph-derived adenomas (58) suggesting a potential non-GH specific role for this receptor in the pituitary. Unlike the case with other examples of endocrine hyperfunction where constitutive activation of the relevant receptor has been described, no intrinsic constitutively active forms of the GHRH receptor have thus far been identified in pituitary adenomas.

Corticotropin-releasing hormone (CRH). The postulated etiology of Cushing's disease has shown tremendous flux since Cushing's original description of the disease (78). The documentation of adrenal hyper-responsiveness to ACTH and the presence of Crooke's hyalinization in the pituitary brought a primary adrenal etiology to the fore. It has been recognized in the last few decades that patients with Cushing's disease may have other associated neuroendocrine abnormalities. Reports of therapeutic response to antiserotoninergic or antidopaminergic agents reverted attention to the hypothalamus (77). Long term follow-up of patients who have undergone trans-sphenoidal resection of microadenomas has indicated recurrence of disease in some patients. A few patients with pituitary Cushing's disease have corticotroph hyperplasia as the cause of the disorder in the absence of a discrete adenoma (75). These findings have implicated CRH excess in the pathogenesis of Cushing's disease (78).

The characterization of CRH in 1981 permitted its identification in a number of extra-pituitary tumors associated with a clinical picture resembling Cushing's

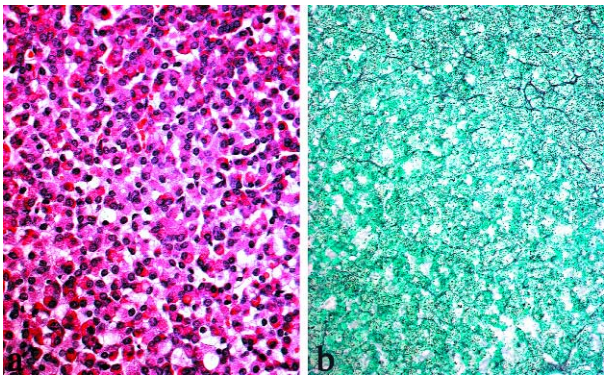


Figure 1. *Pituitary adenomas in mice transgenic for GH-releasing hormone (GHRH).* **a**) A transgenic mouse pituitary reveals mammosomatotroph hyperplasia with prominence of large acidophilic cells (top left) that surrounds an area of disrupted architecture. The tumor lacks acinar structures and is composed of atypical cells with variable cytoplasmic acidophilia and occasional binucleate forms. **b**) The Gordon-sweet silver stain confirms the presence of distended acini in the hyperplastic pituitary (top left) and loss of the reticulin fiber network in the adenoma.

disease; some of these patients had corticotroph hyperplasia (27, 43). In one instance, a hypothalamic gangliocytoma producing CRH was associated with corticotroph hyperplasia and Cushing's disease (14). Continuous infusion of CRH leads to corticotroph hyperplasia (11, 45, 97), but as of yet, has not been shown to result in true adenoma formation.

CRH treatment of pituitary adenomas *in vitro* induces POMC and ACTH mRNA gene expression (138) which are inhibited by dexamethasone (139, 140). Additionally, CRH receptor expression appears not only intact in ACTH-producing pituitary adenomas but, unlike in the rat pituitary, is up-regulated in response to CRH treatment (124). There is currently no evidence of constitutive activation of CRH receptors in corticotroph adenomas. The closely related vasopressin V3 receptor is also intact but may be over-expressed in some corticotroph adenomas where it may play a role in tumor development (30).

Thyrotropin-releasing hormone (TRH). Primary hypothyroidism is a well recognized cause of pituitary thyrotroph and lactotroph hyperplasia. These patients exhibit the spectrum of hyperplasia-to-neoplasia (46, 130), suggesting that continuous stimulation by TRH may lead to thyrotroph adenoma (60). TRH has also been shown to be expressed in the pituitary (83, 94, 110) and by the different types of pituitary adenomas (80-82, 153).

TRH signaling appears to be intact in pituitary adenomas as evidenced by intact binding and release of

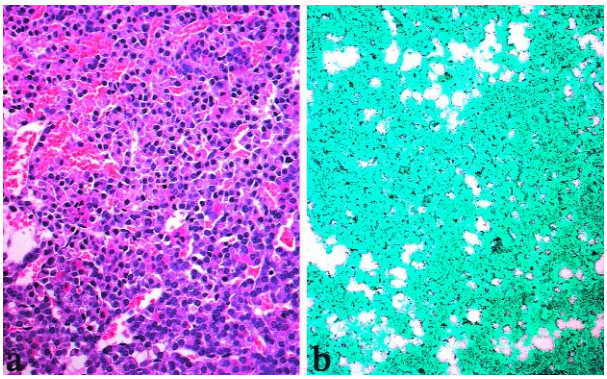


Figure 2. *Anterior pituitary hyperplasia in D2 Receptor-Deficient Mice.* **a**) The pituitary of a dopamine D2R deficient mouse contains a lactotroph adenoma with striking peliosis and nuclear atypia. **b**) A reticulin stain confirms total breakdown of the reticulin fiber network, confirming the development of adenoma superimposed on the underlying lactotroph hyperplasia that younger animals exhibit (not shown).

TSH and PRL (82, 153). TRH receptor expression (152) and structure is grossly unaltered even in thyrotroph adenomas(31). Messenger RNA is, however, alternatively spliced in some pituitary tumors (152). Deletion of exon 3 results in a truncated product which does not bind TRH nor is it activated by it. The relatively higher levels of the truncated forms compared to the full length form of the TRH receptor in lactotroph adenomas (152) may explain some of the paradoxical *in vivo* responses to TRH administration (34).

Gonadotrophin-releasing hormone (GnRH). The occurrence of gonadotroph adenomas in patients with hypogonadism has suggested that the chronic stimulation resulting from primary gonadal failure may play a role in the formation and growth of these adenomas (107, 134). Nevertheless, the majority of gonadotroph adenomas are not associated with underlying primary hypogonadism nor is there evidence of chronic hypothalamic stimulation in the adjacent nontumorous adenohypophysis (123).

Both GnRH (108, 126) and GnRH receptor expression have been documented in the different types of pituitary adenomas (99, 137). Furthermore, pituitary adenomas with truncated GnRH receptors have been described that fail to respond to GnRH stimulation by enhancing calcium transport and gonadotrophin release *in vitro* (7). No activating mutations of the GnRH receptor, including the exon 3 “hot spot,” have been identified.

Other releasing factors. The pathogenesis of lactotroph adenomas may involve defects in inhibitory hypothalamic factors or excessive stimulation by a putative PRL-releasing factor such as TRH or vasoactive intestinal peptide (VIP) (103). The presence of lactotroph hyperplasia in the tissue surrounding lactotroph adenomas in some cases (15, 123) would be consistent with this theory. Moreover, lactotrophs normally proliferate during pregnancy (15, 131) thereby implicating estrogen as a candidate factor. Administration of oral contraceptives was implicated in the rapid increase in size and secretion of some lactotroph adenomas (55). The current cumulative clinical experience, however, has refuted this association with little evidence that low doses of oral contraceptives play a significant role in pituitary tumor development.

Loss of inhibitory hormones. *Dopamine.* The role of diminished hypothalamic inhibition was first suggested based on the observation of neovascularization in lactotroph adenomas. It was speculated that neovascularization would allow lactotrophs to escape from tonic dopaminergic inhibition (128). Dopamine signal transduction is mediated through D1 receptors which stimulate adenylyl cyclase activity and D2 receptors (D2R) which inhibit this enzyme. The family of dopamine receptors is much more complex in terms of biochemical, physiological, and pharmacological diversity (132, 143, 151). Nevertheless, it appears that the predominant anterior pituitary dopamine receptor is the D2R (74, 151). Activation of the D2R results in dysregulated cAMP production, potassium and calcium channel fluxes, phosphatidyl inositol turnover, and intracellular calcium concentrations (132). Treatment with EGF or TRH, which stimulates p44/42 MAP kinase, rescues GH3 cells from dopamine-induced apoptosis, with concomitant inhibition of dopamine-induced p38 MAP kinase activation (67). These findings are consistent with the view that dopamine induces apoptosis through p38 MAPK activation, and that the p44/42 MAP kinase signaling through growth factors has an opposing effect on p38 MAPK as well as on apoptosis.

Selective elimination of D2R activity in D2R knockout mice results in lactotroph hyperplasia (73) and, subsequently, lactotroph adenoma formation (10) in female D2R-deficient mice at 17 to 20 months of age (Figure 2). Interestingly, these lesions are monohormonal PRL-immunoreactive neoplasms that display characteristic juxtanuclear Golgi pattern of PRL staining and loss of the reticulin fiber network. Several of these adenomas have been noted to be much larger than normal glands with marked suprasellar extension and invasion of brain but no gross evidence of distant metastases. In contrast, the formation of adenomas in male D2R-deficient mice

without pre-existing or concomitant hyperplasia (10) suggests that prolonged loss of dopamine inhibition may cause neoplastic transformation by a distinct cellular mechanism than in female animals.

While some pituitary tumors have been shown to be responsive to dopamine supression (41, 135), the dopaminergic resistance that is found in some of these tumors implicates diminished DR activity as a putative etiological factor in pituitary tumorigenesis (24). Thus far, however, investigation of the D2R gene has revealed it to be structurally intact in human lactotroph adenomas as well as in adenomas that secrete GH or TSH (44).

Somatostatin. GH secretion is under opposing influence from hypothalamic stimuli including GH-releasing hormone (GHRH), which stimulates, and somatostatin (SS), which inhibits GH secretion. Specific receptors for SS (SSTRs) are expressed on somatotroph adenomas. Earlier studies suggested a relationship between the density of SS receptors on GH tumors and the secretory response to this analogue both *in vitro* and *in vivo* (72, 120). Binding sites for SS, however, have been identified by autoradiography in tumors resistant to the GH-lowering effects of octreotide (23). These findings are consistent with differential adenylyl cyclase coupling by the five known subtypes of SSTRs and their heterogenous expression in pituitary adenomas (98). Expression of SS itself in large invasive GH tumors appears to be diminished compared to that in the normal pituitary (66, 86, 110, 111). Taken together, these findings suggest multiple paracrine, autocrine, as well as endocrine mechanisms for SS-mediated control of somatotroph function and proliferation.

Glucocorticoid hormones. As with other pituitary axes, primary adrenal insufficiency, with prolonged glucocorticoid deficit leads to corticotroph hyperplasia and rarely early adenoma formation (129). A role for CRH in mediating this cell proliferation cannot be excluded.

Lack of suppressibility of corticotroph adenomas by glucocorticoids had been suggested as a possible mechanism involved in the pathological ACTH secretion in Cushing's disease and Nelson's syndrome (91). The human glucocorticoid receptor (GCR) pre-mRNA is alternatively spliced to generate a GCR α isoform and the N-terminally closely related β isoform (18). The β isoform, however, differs in its 50 amino acid C-terminus which contains a unique 15-amino acid sequence that hinders glucocorticoid binding and gene transactivation (18). The functional inter-relationship between the two GCR isoforms in the pituitary and pituitary adenomas will undoubtedly be the focus of future studies. Nevertheless, a molecular basis for glucocorticoid insensitivity has already been described in association with generalized or selective loss of function (18). Specific point mutations resulting in diminished ligand binding in the glucocorticoid hormone-binding domain are now known in cases of familial glucocorticoid resistance (61). Additionally, a novel germ line mutation has been reported to result in pituitary Cushing's disease (68). Similarly, rare reports of somatic mutations in the GCR with diminished glucocorticoid inhibition were noted in Nelson's syndrome (69) and as predicted in cases of ectopic Cushing's syndrome (119).

Thyroid hormones. The development of pituitary thyrotroph adenomas in patients with prolonged primary hypothyroidism has provided further evidence supporting the hypothalamic role in pituitary tumorigenesis (60, 130).

Thyroid hormones mediate their actions via nuclear thyroid hormone receptors (TRs) that bind to specific regulatory hormone response elements (47, 144). There are two major classes of TRs, designated as α and β, each of which undergoes alternative splicing to generate α1 and α2 and β1 and β2 isoforms (47, 144). With the exception of the β2 form, which is predominantly expressed in the hypothalamic-pituitary system, these receptor isoforms are ubiquitously expressed. Of interest in the pituitary, the β1 and β2 isoforms appear to be expressed to a lesser extent in endocrinologically-inactive adenomas compared with the normal gland (47, 144). Screening TRα mRNA identified three novel missense mutations, two in the common TRα region and another that was α2 specific. TRβ response elements failed to show any differences from published sequences (96).

In contrast, mice with targeted disruption of the entire TR-β locus exhibit elevated thyroid hormone levels as a result of abnormal central regulation of thyrotropin but do not develop pituitary tumors (3). Thus, the putative differential hormone regulatory and mitogenic effects of the different THR isoforms in the pituitary remains largely understood.

Gonadal steroids. The development of pituitary gonadotroph adenomas in patients with prolonged primary hypogonadism suggests that the lack of hormone negative feedback may facilitate pituitary tumor formation (107, 134). Again, however, the role of GnRH stimulation cannot be easily distinguished from that of gonadal hormone inhibition in the development of these adenomas.

Growth factors and receptors.

Growth factors are polypeptides of several major

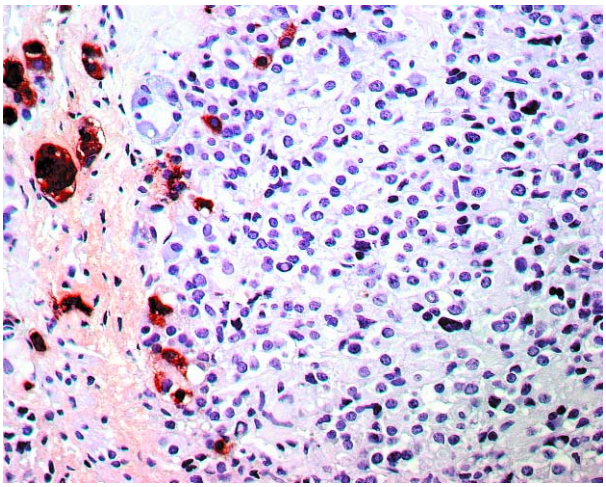


Figure 3. *Lack of expression of human follistatin mRNA by gonadotroph adenomas.* Follistatin is localized in pituitary gonadotrophs of the nontumorous adenohypophysis (left). In contrast, a gonadotroph adenoma (right) is totally devoid of follistatin mmunoreactivity. A few trapped nontumorous cells within the capsule of the adenoma are positive.

families that regulate cell replication and functional differentiation by directly altering the expression of specific genes (121). They are considered to play an important role in the multistep pathway of tumorigenesis. A number of oncogene products are homologous to growth factors, their receptors, or enzymes that participate in the mitogenic process. In several systems, growth factors have been shown to interact with specific membrane receptors in regulating cell growth and gene expression in an autocrine or paracrine manner. Some are known to affect hormone production and some are, in turn, modulated by hormones (36). A few have been identified in the hypothalamus and are considered to play a physiological role in pituitary regulation (12).

The pituitary is a site of both synthesis and action of growth factors (36, 145). A number of growth factors have been identified in adenohypophysial cells, including insulin-like growth factors-I and -II (IGF-I, IGF-II), epidermal growth factor (EGF), nerve growth factor (NGF) (109), transforming growth factor-α (TGF-α), transforming growth factor-β (TGF-β), and basic fibroblast growth factor (bFGF). Several partially characterized pituitary-derived growth factors have also been described (36, 145), including thyroid hormone-inducible growth factor, mammary cell growth factor (106), adrenal growth factor (125), chondrocyte growth factor (65, 70), and adipocyte growth factor. Growing evidence suggests that human pituitary tumor cells produce multiple peptides which stimulate rat adenohypophysial cell replication *in vitro* (146). The relative significance of these different growth factors in human pituitary adenomas remains to be established, however, several have been implicated in the pathogenesis of these tumors.

The epidermal growth factor family. The EGF family of ligands includes EGF, TGF-α, amphiregulin, heparin-binding EGF-like growth factor (HB-EGF), and betacellulin (BTC) (22). An additional family of EGF-related agonists include neuregulins which include glial growth factors (GGFs), neu differentiation factors (NDFs)/heregulins, ligands for erbB-3 and erbB-4 (22). Of interest, GGFs were purified from the bovine pituitary (92). It is currently not known, however, which specific subsets of erbB receptors become activated in response to each of these ligands.

Transforming growth factor-α. Transforming growth factor-α is expressed as a membrane-anchored protein by human adenohypophysial cells and tumors (38). TGF-α may alter pituitary production of GH, PRL, TSH as well as cell proliferation (42). Estrogen stimulation has been implicated in pituitary tumorigenesis (76) and TGF-α appears to mediate some estrogenic effects (105). Targeted overexpression of TGF-α under the control of the PRL promoter results in lactotroph adenomas (95) providing compelling evidence for the significance of this growth factor in pituitary tumorigenesis.

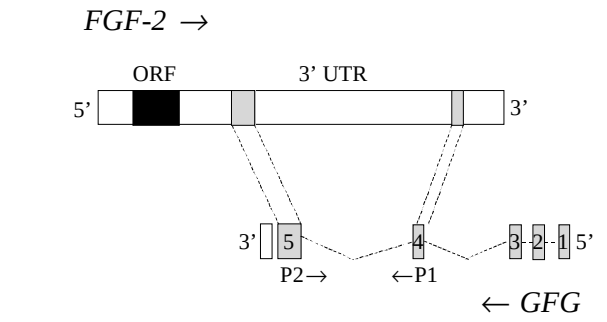
Epidermal growth factor and receptor (EGF; EGF-R). EGF is detectable by immunohistochemistry in most adenohypophysial cells and its mRNA is expressed with marked variation in all types of functional and nonfunctional adenomas (84). EGF potently stimulates PRL (104, 118, 149) and ACTH secretion (28, 115) and has been reported to stimulate (62) and inhibit (36) GH secretion by nontumorous rat pituitary cells *in vitro*. The selective expression and specific effects of EGF suggest that the pituitary is an important target site for this growth factor's action.

The common receptor of EGF and TGF-α, EGF-R, is a 170-kD plasma membrane tyrosine kinase product of the protooncogene v-*erb*B. EGF-R is overexpressed in several types of human cancers and in most instances this overexpression is accompanied by TGF-α expression; expression of this receptor appear to correlate with tumor aggressiveness. EGF-R is expressed by pituitary adenomas with the highest levels detected in recurrent somatotroph adenomas and aggressive silent subtype 3 adenomas, suggesting a selective mechanism for the EGF/EGF-R family in the growth of aggressive somatotroph tumors (84). The importance of the EGF-R in

the somatotroph has been further addressed recently in transgenic mice using expressing a dominant negative EGF-R mutant lacking the intracellular protein kinase domain (EGFR-tr). Directed EGFR-tr expression to GH- and PRL- producing cells resulted in dwarf mice with hypoplasia of these cell populations (122). When EGF-R-tr over-expression was delayed to the postnatal period, however, no specific phenotype was observed. These findings point to EGF-R as an integral component in the differentiation of the somatotroph.

The EGF-R is one of four highly homologous tyrosine kinase receptors which include erbB2/HER2/neu/p185, erbB-3 (HER3), and erbB-4 (HER4) (114). Growing evidence in support of functional cross-talk between the different members of this receptor family is now well recognized (117). Ligand-induced stimulation can result in transphosphorylation of *neu* via EGF-R (32, 117). Over-expression of a wild type EGF-R and heterocomplex formation with *neu* dramatically increases receptor autophosphorylation and binding of EGF (50, 117). Cytoplasmic positivity for *neu* can be identified in nontumorous pituitary cells using an antibody to the intracytoplasmic domain of neu. No membrane staining is found using an antibody to the extracellular domain; the latter is said to reflect gene amplification (39). *Neu* mRNA expression has been described in the normal and tumorous pituitary. No differences in degrees of mRNA expression, however, have been noted between the different adenoma types and normal human pituitary tissue as examined by competitive PCR (39). As *neu* can be activated to an oncogene by a point mutation in the transmembrane region, nucleotide substitutions in this domain were investigated. Direct sequencing of codon 659 revealed no point mutations in any of the tumors. Moreover, since amplification of *neu* has been noted in a number of human malignancies, DNA from these pituitary adenomas was examined by differential PCR. No detectable differences were noted between the *neu* gene and the single-copy reference gene INF-γ. These findings indicated that the *neu* gene is expressed in a homogenous pattern in pituitary cells and their adenomas but that this expression is not associated with gene amplification or activating mutations to suggest a direct role in pituitary tumorigenesis.

The transforming growth factor-β family. Transforming growth factor (TGF)-β has been implicated in the regulation of normal and neoplastic cell function. TGF-beta regulates the expression of various proteins, including p27Kip1 (p27), a cell cycle inhibitory protein. TGF-beta 1/2/3 isoforms and the TGF-beta-



a

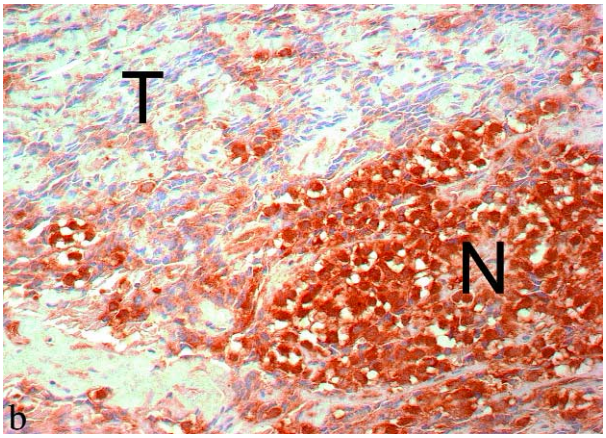
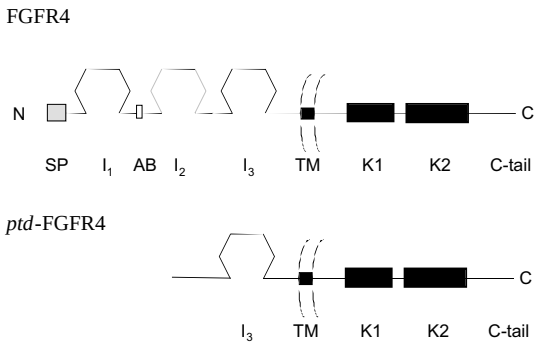


Figure 4. *The FGF antisense gene GFG.* **a**) Gene structure and regions of complimentarity between the mammalian sense FGF and antisense GFG genes are depicted. **b**) By immunohistochemistry, nontumorous pituitary (N, lower right) exhibits cytoplasmic GFG protein (brown); an infiltrative pituitary tumor (T, upper left) exhibits reduced cytoplasmic staining for GFG.

receptor are expressed in normal and adenomatous pituitaries (64). Dispersed pituitary adenomas cells show a biphasic response to TGF-β with changes in FSH secretion. The TGF-β family, however, is represented in at least three different forms in the pituitary. Inhibins and activins consist of two homo- or heterodimeric polypeptide subunits derived from a common precursor (156); inhibin A (α-βA) and inhibin B (α-βB) selectively inhibit the release of FSH from pituitary gonadotroph cells whereas activin (βA-βB), activin A (βA-βA) and activin B (βB-βB) stimulate its release. Inhibin subunits are expressed by pituitary gonadotroph adenomas (8, 56) and activin is known to stimulate hormone secretion by these tumors (6). Activin effects are mediated by two kinds of binding proteins, activin receptors and follistatin (156); the former are required for activin binding, but follistatin binds the protein resulting in decreased activity. Activin receptors are expressed in gonadotroph adenomas and follistatin expression is reduced or absent



a

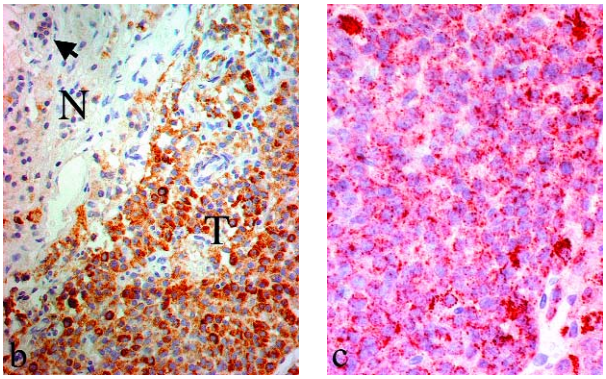


Figure 5. *Characterization of ptd-FGFR4 protein in the human pituitary.* **a**) Full length FGFR4 is composed of a signal peptide (SP), three extracellular Ig-like domains (I_1, I_2 and I_3) with an acid box (AB) between I_1 and I_2, a transmembrane domain (TM), a split kinase (K1 and K2) and a C terminal tail (C). In contrast, ptd-FGFR4 initiates transcription in exon 5, thereby lacking a signal peptide and the first 2 Ig-like domains. This results in cytoplasmic localization of this N-terminally truncated receptor. **b**) Immunohistochemistry localizes C-terminal FGFR4 immunoreactivity in the cytoplasm of human pituitary tumor (T, bottom right) but not in normal pituitary cells (N, top left). **c**) Transgenic mice expressing ptd-FGFR4 under the control of the mouse prolactin promoter develop pituitary lactotroph adenomas that contain cytoplasmic immunoreactivity for C-terminal FGFR4, similar to human tumors.

in some (Figure 3) (112), suggesting the possibility of enhanced activin stimulation as a pathogenetic mechanism in the development of these common pituitary tumors. In contrast, activin treatment inhibited cell proliferation in some non-functioning primary human tumors with diminished follistatin mRNA expression (29). These findings suggest a functional but heterogenous role for the activin/follistatin balance in modulating pituitary tumor cell replication.

Vascular endothelial growth factor. Vascular endothelial growth factor (VEGF) also known as vascular permeability factor (VPG) was also one of the growth factors first isolated from the pituitary (53). VEGF exists in a number of isoforms in human and rodent tissues including VEGF206h/205r, VEGF189h/188r, VEGF165h/164r, VEGF145h/144r and VEGF121) that differ in their molecular masses and biological activities. The VEGF isoforms bind with two tyrosine-kinase receptors, KDR/flk-1 and flt-1. In addition, VEGF165 binds with co-receptor, neuropilin-1, which is expressed in human endothelial cells and several types of non-endothelial cells including solid tumors. Recent studies on the role of estrogen in the regulation of tumor angiogenesis demonstrated that this steroid induces neovascularization in parallel with early induction of VEGF and the VEGFR2- (flk-1/KDR) protein expression in both blood vessels and non-endothelial cells (19). Moreover, estrogen-induced rat pituitary tumors in Fisher 344 rats express higher VEGF164 and neuropilin-1 levels compared to control untreated rat pituitaries (20). These findings suggest that over-expression of VEGF and its receptor (VEGFR-2) may play an important role in the early phases of estrogen induced tumor angiogenesis in the rat pituitary.

Fibroblast growth factors and receptors. *Fibroblast growth factors (FGFs)*. Basic fibroblast growth factor (bFGF, also known as FGF-2) is one of an ever-expanding family of FGFs several of which possess mitogenic, angiogenic, and hormone regulatory functions (93). bFGF immunoreactivity was described originally in the non-hormone producing bovine pituitary folliculo-stellate cells (54); since bFGF has been shown to regulate GH, PRL and TSH secretion by the rodent pituitary (17, 79), it was implicated in paracrine regulation within the pituitary. In the human pituitary, in contrast, bFGF is produced by adenohypophysial cells that comprise pituitary adenomas (37, 63, 87). Pituitary-derived bFGF stimulates replication of PRL-secreting cells (116). Elevated circulating bFGF-like immunoreactivity is noted in patients with multiple endocrine neoplasia (MEN)-1 (157) and in patients with sporadic pituitary adenomas (37).

The FGF-related *hst* has been found in transforming DNA of human PRL-secreting tumors (51) and transfection studies have shown that hst facilitates lactotroph proliferation *in vivo* and *in vitro* (133). Moreover, in one mouse model, estrogen-induced pituitary tumorigenesis was associated with parallel increases in the expression of a pituitary tumor transforming gene (PTTG) as well as FGF-2 (59). Transgenic mice expressing FGF-2 under the control of the GH and the α-subunit promot-

ers developed hyperplasia of several adenohypophysial cell types but not frank adenomatous changes (147).

In *Xenopus laevis* oocytes, a 1.5 kb FGF-2 antisense (GFG) RNA complementary to the third exon and 3'UTR of FGF-2 mRNA has been implicated in FGF-2 mRNA regulation. The human homolog has been localized to the same chromosomal site as FGF-2 (chromosome 4, JO4513 adjacent to D4S430), confirming this as a human endogenous anti-sense gene (16). This GFG anti-sense gene also encodes a 35 Kd protein, which is highly homologous with the MutT family of antimutator NTPases. Pituitary tumors have been shown to express FGF-2 and GFG while the normal human pituitary expresses GFG but not FGF-2; GFG protein levels are higher in the normal gland than in most tumors (Figure 4). Aggressive pituitary adenomas appear to express more FGF-2 than GFG mRNA. Expression of GFG in transfected GH4 mammosomatotroph cells results in enhanced PRL gene expression and protein translation (16). Moreover, despite the fact that GFG expression does not down regulate pituitary FGF-2 mRNA expression, GFG expression inhibits pituitary cell proliferation. Taken together, these recent findings suggest that the GFG-encoded protein may represent a novel mechanism involved in restraining pituitary tumor cell growth while promoting hormonal activity.

Fibroblast growth factor receptors (FGFRs). There are 4 mammalian FGFR genes encoding a complex family of transmembrane receptor tyrosine kinases (RTKs) (48). Each prototypic receptor is composed of 3 immunoglobulin (Ig)-like extracellular domains, 2 of which are involved in ligand binding, a single transmembrane domain, a split tyrosine kinase, and a COOH-terminal tail with multiple autophosphorylation sites (48). Multiple forms of cell-bound or secreted receptors are produced by the same gene. Tissue-specific alternative splicing, variable polyadenylation sites and alternative initiation of translation result in truncated receptor forms (113, 155). The first two extracellular loops of FGFR1 can be secreted as soluble circulating FGF binding proteins (57), but their physiological importance remains to be established. Different FGFRs can dimerize, so that truncated forms of FGFR1 block signalling through FGFR1, 2, and 3 (148).

Structural alterations of FGFRs may play a role in human tumorigenesis. For example, FGFR1 is highly expressed in the brain (52) but the shorter (2 Ig-domain) form of FGFR1 is more abundant in some CNS glioblastomas (33). Anti-sense targeted interruption of FGFR1 reduces malignant melanoma cell proliferation and differentiation (21). FGFR2 exon switching has

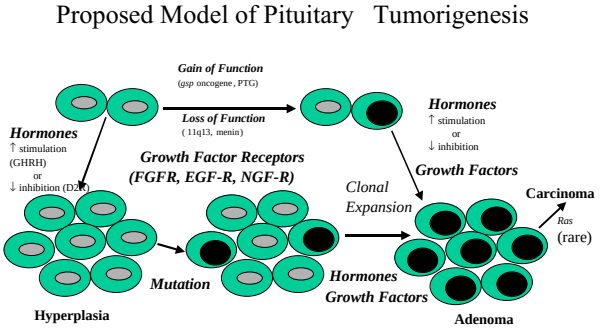


Figure 6. *Proposed model of pituitary tumorigenesis.* An integrated approach considers hypothalamic influence of stimulation or loss of negative feedback inhibition contributing to an intrinsic pituitary defect in cell cycle regulation. Animal models and patients with hypophysiotrophic hormone excess, suppressive hormone insufficiency, or growth factor excess develop hyperplasia (left pathway); the increased proliferative activity predisposes to genomic instability (cells with dark nuclei) and subsequent adenoma formation. Most human pituitary adenomas are unassociated with hyperplasia and likely result from a rapid early progressive genetic event which alters cell cycle control (dark nucleus) rendering these cells targets for further stimulation by hormonal and/or growth factor signals (right).

been observed to accompany prostate cell transformation (154). The normal pituitary expresses mRNAs for FGFR 1, 2 and 3. An interesting finding was the documentation of novel truncated mRNAs for the first and second Ig-like loops of FGFR4 in the nontumorous pituitary and a kinase-containing variant of FGFR4 with an alternative transcription initiation site in pituitary adenomas (Figure 5) (1, 2). This tumor-derived kinase containing FGFR4 isoform is transforming *in vitro* and *in vivo* and has been recently shown to result in lactotroph adenomas in transgenic mice (40).

Taken together, these data suggest that dysregulated FGF/FGFR function plays a role in pituitary tumorigenesis and that FGFR4 is a candidate tumor-specific kinase.

The nerve growth factor family. NGF overexpression targeted to lactotrophs results in dramatic hyperplasia of those cells, however, tumor formation has not been demonstrated (26). Further, treatment of human prolactinoma cells with NGF results in decreased proliferation *in vitro*, reduced capacity to form colonies in soft agar, and loss of tumorigenic activity in nude mice (100). NGF appears to induce D2 receptor expression in human prolactinomas(100)and directs differentiation of bihormonal GH3 cells into mature lactotrophs with D2 receptor expression (101). Moreover, gp140trk and gp75 components of of the NGF receptor are expressed

in responder prolactinoma cell lines. NGF anti-sense treatment results in loss of expression of D2 receptors and an increase cell proliferation (102). Aberrant expression of NGF may, therefore, contribute to unrestrained cell proliferation and/or diminished responsiveness to dopamine agonist treatment.

Conclusions

Pituitary adenomas are common neoplasms that exhibit a wide range of biologic behavior. Numerous factors have been shown to govern pituitary cell proliferation; these various hypophysiotropic hormones and growth factors likely play a role as promoters of tumor cell growth in genetically transformed cells (Figure 6). In some instances, abnormal forms of growth factor receptors maybe important in the early stages of cell transformation consistent with the clonal composition of pituitary adenomas.

References

1. Abbass SAA, Asa SL, Ezzat S (1997) Altered expression of fibroblast growth factor receptors in human pituitary adenomas. *J Clin Endocrinol Metab* 82: 1160-1166
2. Abbass SAA, Ezzat S, Guha A, Asa SL (1999) Novel isoforms of fibroblast growth factor receptor 4 (FGFR4) are expressed by central nervous system neoplasms. *Endo Soc Mtg* 549 (Abstract)
3. Abel ED, Boers ME, Pazos-Moura C, Moura E, Kaulbach H, Zakaria M, Lowell B, Radovick S, Liberman MC, Wondisford F (1999) Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system. *J Clin Invest* 104: 291-300
4. Adams EF, Bhuttacharji SC, Halliwell CLJ, Loizou M, Birch G, Mashiter K (1984) Effect of pancreatic growth hormone releasing factors on GH secretion by human somatotrophic pituitary tumours in cell culture. *Clin Endocrinol* (Oxf) 21: 709-718
5. Adams EF, Winslow CLJ, Mashiter K (1983) Pancreatic growth hormone releasing factor stimulates growth hormone secretion by pituitary cells. *Lancet* 1: 1100-1101
6. Alexander JM, Jameson JL, Bikkal HA, Schwall RH, Klibanski A (1991) The effects of activin on follicle-stimulating hormone secretion and biosynthesis in human glycoprotein hormone-producing pituitary adenomas. *J Clin Endocrinol Metab* 72: 1261-1267
7. Alexander JM, Klibanski A (1994) Gonadotropin-releasing hormone receptor mRNA expression by human pituitary tumors *in vitro*. *J Clin Invest* 93: 2332-2339
8. Alexander JM, Swearingen B, Tindall GT, Klibanski A (1995) Human pituitary adenomas express endogenous inhibin subunit and follistatin messenger ribonucleic acids. *J Clin Endocrinol Metab* 80: 147-152
9. Asa SL. Tumors of the Pituitary Gland. Third Series(Fascicle 22). (1998) Washington, D.C., Armed Forces Institute of Pathology. *Atlas of Tumor Pathology*. Rosai J. Ref Type: Serial (Book, Monograph)
10. Asa SL, Kelly MA, Grandy DK, Low MJ (1999) Pituitary lactotroph adenomas develop after prolonged lactotroph hyperplasia dopamine D2 receptor-deficient mice. *Endocrinology* 140: 5348-5355
11. Asa SL, Kovacs K, Hammer GD, Liu B, Roos BA, Low MJ (1992) Pituitary corticotroph hyperplasia in rats implanted with a medullary thyroid carcinoma cell line transfected with a corticotropin-releasing hormone complementary deoxyribonucleic acid expression vector. *Endocrinology* 131: 715-720
12. Asa SL, Kovacs K, Melmed S (1995) The hypothalamic-pituitary axis. In: Melmed S (ed) *The Pituitary*. Blackwell Scientific Publication Inc., Boston,3-44
13. Asa SL, Kovacs K, Stefaneanu L, Horvath E, Billestrup N, Gonzalez-Manchon C, Vale W (1992) Pituitary adenomas in mice transgenic for growth hormone-releasing hormone. *Endocrinology* 131: 2083-2089
14. Asa SL, Kovacs K, Tindall GT, Barrow DL, Horvath E, Vecsei P (1984) Cushing's disease associated with an intrasellar gangliocytoma producing corticotrophin-releasing factor. *Ann Intern Med* 101: 789-793
15. Asa SL, Penz G, Kovacs K, Ezrin C (1982) Prolactin cells in the human pituitary. A quantitative immunocytochemical analysis. *Arch Pathol Lab Med* 106: 360-363
16. Asa SL, Ramyar L, Murphy PR, Li AW, Ezzat S (2001) The endogenous fibroblast growth factor-2 antisense gene product regulates pituitary cell growth and hormone production. *Mol Endocrinol* 15: 589-599
17. Baird A, Mormède P, Ying S-Y, Wehrenberg WB, Ueno N, Ling N, Guillemin R (1985) A nonmitogenic pituitary function of fibroblast growth factor: regulation of thyrotropin and prolactin secretion. *Proc Natl Acad Sci USA* 82: 5545-5549
18. Bamberger CM, Schulte HM, Chrousos GP (1996) Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocr Rev* 17: 245-261
19. Banerjee SK, Sarkar DK, Weston AP, De A, Campbell DR (1997) Over expression of vascular endothelial growth factor and its receptor during the development of estrogen-induced rat pituitary tumors may mediate estrogen-initiated tumor angiogenesis. *Carcinogenesis* 18: 1155-1161
20. Banerjee SK, Zoubine MN, Tran TM, Weston AP, Campbell DR (2000) Overexpression of vascular endothelial growth factor164 and its co- receptor neuropilin-1 in estrogen-induced rat pituitary tumors and GH3 rat pituitary tumor cells. *Int J Oncol* 16: 253-260
21. Becker D, Lee PLP, Rodeck U, Herlyn M (1992) Inhibition of the fibroblast growth factor receptor 1 (FGFR-1) gene in human melanocytes and malignant melanomas leads to inhibition of proliferation and signs indicative of differentiation. *Oncogene* 7: 2303-2313
22. Beerli RR, Hynes NE (1996) Epidermal growth factor-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities. *J Biol Chem* 271: 6071-6076

23. Bertherat J, Chanson P, Dewailly D, Dupuy M, Jaquet P, Peillon F, Epelbaum J (1993) Somatostatin receptors, adenylate cyclase activity, and growth hormone (GH) response to octreotide in GH-secreting adenomas. *J Clin Endocrinol Metab* 77: 1577-1583
24. Bevan JS, Burke CW, Esiri MM, Adams CBT, Ballabio M, Nissim M, Faglia G (1989) Studies of two thyrotrophin-secreting pituitary adenomas: evidence for dopamine receptor deficiency. *Clin Endocrinol* (Oxf) 31: 59-70
25. Billestrup N, Swanson LW, Vale W (1986) Growth hormone-releasing factor stimulates proliferation of somatotrophs *in vitro*. *Proc Natl Acad Sci USA* 83: 6854-6857
26. Borrelli E, Sawchenko PE, Evans RM (1992) Pituitary hyperplasia induced by ectopic expression of nerve growth factor. *Proc Natl Acad Sci USA* 89: 2764-2768
27. Carey RM, Varma SK, Drake CR, Jr., Thorner MO, Kovacs K, Rivier J, Vale W (1984) Ectopic secretion of corticotropin-releasing factor as a cause of Cushing's syndrome. A clinical, morphologic, and biochemical study. *N Engl J Med* 311: 13-20
28. Childs GV, Rougeau D, Unabia G (1995) Corticotropin-releasing hormone and epidermal growth factor: mitogens for anterior pituitary corticotropes. *Endocrinology* 136: 1595-1602
29. Danila DC, Inder WJ, Zhang X, Alexander JM, Swearingen B, Hedley-Whyte ET, Klibanski A (2000) Activin effects on neoplastic proliferation of human pituitary tumors. *J Clin Endocrinol Metab* 85: 1009-1015
30. De Keyzer Y, Rene P, Lenne F, Auzan C, Clauser E, Bertagna X (1997) V3 vasopressin receptor and corticotropic phenotype in pituitary and nonpituitary tumors. *Horm Res* 47: 259-262
31. Dong Q, Brucker-Davis F, Weintraub BD, Smallridge RC, Carr F, Battey J, Spiegel AM, Shenker A (1996) Screening of candidate oncogenes in human thyrotroph tumors: absence of activating mutations of the Gαq, Gα11, Gαs, or thyrotropin-releasing hormone receptor genes. *J Clin Endocrinol Metab* 81: 1134-1140
32. Dougall WC, Quan X, Peterson NC, Miller MJ, Samanta A, Greene MI (1994) The *neu*-oncogene: signal transduction pathways, transformation mechanisms and evolving therapies. *Oncogene* 9: 2109-2123
33. Eisemman A, Ahn AJ, Graziani G, Tronick SR, Ron D (1991) Alternative splicing generates at least five different isoforms of the human bFGF receptor. *Oncogene* 6: 1195-1202
34. Ezzat S (1992) Hypophysiotropic regulation of anterior pituitary hormones: cellular and molecular mechanisms. In: Selman WR (ed) *Neuroendocrinology*. Williams & Wilkins, Baltimore,3-18
35. Ezzat S, Asa SL, Stefaneanu L, Whittom R, Smyth HS, Horvath E, Kovacs K, Frohman LA (1994) Somatotroph hyperplasia without pituitary adenoma associated with a long standing growth hormone-releasing hormone-producing bronchial carcinoid. *J Clin Endocrinol Metab* 78: 555-560
36. Ezzat S, Melmed S (1990) The role of growth factors in the pituitary. *J Endocrinol Invest* 13: 691-698
37. Ezzat S, Smyth HS, Ramyar L, Asa SL (1995) Heterogeneous *in vivo* and *in vitro* expression of basic fibroblast growth factor by human pituitary adenomas. *J Clin Endocrinol Metab* 80: 878-884
38. Ezzat S, Walpola IA, Ramyar L, Smyth HS, Asa SL (1995) Membrane-anchored expression of transforming growth factor-a in human pituitary adenoma cells. *J Clin Endocrinol Metab* 80: 534-539
39. Ezzat S, Zheng L, Smyth HS, Asa SL (1997) The c-*erb*B-2/*neu* proto-oncogene in human pituitary tumours. Clin Endocrinol (Oxf) 46: 599-606
40. Ezzat S, Zheng L, Zhu X-F, Wu GE, Asa SL. Pituitary tumorigenesis induced by a novel transforming isoform of fibroblast growth factor receptor 4. (2001) Submitted
41. Filetti S, Rapoport B, Aron DC, Greenspan FC, Wilson CB, Fraser W (1982) TSH and TSH-subunit production by human thyrotrophic tumour cells in monolayer culture. *Acta Endocrinol (Copenh)* 99: 224-231
42. Fisher DA, Lakshmanan J (1990) Metabolism and effects of epidermal growth factor and related growth factors in mammals. *Endocr Rev* 11: 418-442
43. Fjellestad-Paulsen A, Abrahamsson P-A, Bjartell A, Grino M, Grimelius L, Hedeland H, Falkmer S (1988) Carcinoma of the prostate with Cushing's syndrome. A case report with immunohistochemical and chemical demonstration of immunoreactive corticotropin-releasing hormone in plasma and tumor tissue. *Acta Endocrinol (Copenh)* 119: 506-516
44. Friedman E, Adams EF, Hoog A, Gejman PV, Carson E, Larsson C, De Marco L, Werner S, Fahlbusch R, Nordenskjöld M (1994) Normal structural dopamine type 2 receptor gene in prolactin-secreting and other pituitary tumors. *J Clin Endocrinol Metab* 78: 568-574
45. Gertz BJ, Contreras LN, McComb DJ, Kovacs K, Tyrrell JB, Dallman MF (1987) Chronic administration of corticotropin-releasing factor increases pituitary corticotroph number. *Endocrinology* 120: 381-388
46. Gesundheit N, Petrick PA, Nissim M, Dahlberg PA, Doppman JL, Emerson CH, Braverman LE, Oldfield EH, Weintraub BD (1989) Thyrotropin-secreting pituitary adenomas: clinical and biochemical heterogeneity. Case reports and follow-up of nine patients. *Ann Intern Med* 111: 827-835
47. Gittoes NJL, McCabe CJ, Verhaeg J, Sheppard MC, Franklyn JA (1997) Thyroid hormone and estrogen receptor expression in normal pituitary and nonfunctioning tumors of the anterior pituitary. *J Clin Endocrinol Metab* 82: 1960-1967
48. Givol D, Yayon A (1992) Complexity of FGF receptors: genetic basis for structural diversity and functional specificity. *FASEB J* 6: 3362-3369
49. Godfrey P, Rahal J, Beamer W, Copeland N, Jenkins N, Mayo K (1993) GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. *Nature Genet* 4: 227-232
50. Goldman R, Levy RB, Peles E, Yarden Y (1990) Heterodimerization of the erbB-1 and erbB-2 receptors in human breast carcinoma cells: A mechanism for receptor transregulation. *Biochem* J 29: 11024-11028

51. Gonsky R, Herman V, Melmed S, Fagin J (1991) Transforming DNA sequences present in human prolactin-secreting pituitary tumors. *Mol Endocrinol* 5: 1687-1695

52. Gonzalez AM, Logan A, Ying W, Lappi DA, Berry M, Baird A (1994) Fibroblast growth factor in the hypothalamic-pituitary axis: Differential expression of fibroblast growth factor-2 and a high affinity receptor. *Endocrinology* 134: 2289-2297

53. Gospodarowicz D, Abraham JA, Schilling J (1989) Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. *Proc Natl Acad Sci USA* 86: 7311-7315

54. Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G (1987) Structural characterization and biological functions of fibroblast growth factor. *Endocr Rev* 8: 95-114

55. Grossman A, Besser GM (1985) Prolactinomas. *Br Med J* 290: 182-184

56. Haddad G, Penabad JL, Bashey HM, Asa SL, Gennarelli TA, Cirullo R, Snyder PJ (1994) Expression of activin/inhibin subunit messenger ribonucleic acids by gonadotroph adenomas. *J Clin Endocrinol Metab* 79: 1399-1403

57. Hanneken A, Ying W, Ling N, Baird A (1994) Identification of soluble forms of the fibroblast growth factor receptor in blood. *Proc Natl Acad Sci USA* 91: 9170-9174

58. Hashimoto K, Koga M, Motomura T, Kasayama S, Kouhara H, Ohnishi T, Arita N, Hayakawa T, Sato B, Kishimoto T (1995) Identification of alternatively spliced messenger ribonucleic acid encoding truncated growth hormone-releasing hormone receptor in human pituitary adenomas. *J Clin Endocrinol Metab* 80: 2933-2939

59. Heaney AP, Horwitz GA, Wang Z, Singson R, Melmed S (1999) Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis. *Nature Medicine* 5: 1317-1321

60. Horvath E, Kovacs K (1991) The adenohypophysis. In: Kovacs K, Asa SL (eds) *Functional Endocrine Pathology*. Blackwell Scientific Publications, Inc., Boston,245-281

61. Hurley DM, Accili D, Stratakis CA, Karl M, Vamvakopoulos N, Rorer E, Constatine K, Taylor SI, Chrousos GP (1991) Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *J Clin Invest* 87: 680-686

62. Ikeda H, Mitsuhashi T, Kubota K, Kuzuya N, Uchimura H (1984) Epidermal growth factor stimulates growth hormone secretion from superfused rat adenohypophyseal fragments. *Endocrinology* 115: 556-558

63. Jin L, Chandler WF, Lloyd RV (1994) Localization of basic fibroblast growth factor (bFGF) protein and mRNA in human pituitaries: Regulation of bFGF mRNA by gonadotropin-releasing hormone. *Endocr Pathol* 5: 27-34

64. Jin L, Qian X, Kulig E, Sanno N, Scheithauer BW, Kovacs K, Young WF, Jr., Lloyd RV (1997) Transforming growth factor-beta, transforming growth factor-beta receptor II, and p27Kip1 expression in nontumorous and neoplastic human pituitaries. *Am J Pathol* 151: 509-519

65. Jones KL, Villela JF, Lewis UJ (1986) The growth of cultured rabbit articular chondrocytes is stimulated by pituitary growth factors but not by purified human growth hormone or ovine prolactin. *Endocrinology* 118: 2588-2593

66. Joubert (Bression) D, Benlot C, Lagoguey A, Garnier P, Brandi AM, Gautron JP, LeGrand JC, Peillon F (1989) Normal and growth hormone (GH)-secreting adenomatous human pituitaries release somatostatin and GH-releasing hormone. *J Clin Endocrinol Metab* 68: 572-577

67. Kanasaki H, Fukunaga K, Takahashi K, Miyazaki K, Miyamoto E (2000) Involvement of p38 mitogen-activated protein kinase activation in bromocriptine-induced apoptosis in rat pituitary GH3 cells. *Biol Reprod* 62: 1486-1494

68. Karl M, Lamberts SWJ, Koper JW, Katz DA, Huizenga NE, Kino T, Haddad BR, Hughes MR, Chrousos GP (1996) Cushing's disease preceded by generalized glucocorticoid resistance: clinical consequences of a novel dominant-negative glucocorticoid receptor mutation. *Proc Assoc Am Physicians* 108: 296-307

69. Karl M, von Wichert G, Kempter E, Katz DA, Reincke M, Mönig H, Ali IU, Stratakis CA, Oldfield EH, Chrousos GP, Schulte HM (1996) Nelson's syndrome associated with a somatic frame shift mutation in the glucocorticoid receptor gene. *J Clin Endocrinol Metab* 81: 124-129

70. Kasper S, Friesen HG (1986) Human pituitary tissue secretes a potent growth factor for chondrocyte proliferation. *J Clin Endocrinol Metab* 62: 70-76

71. Kawakita S, Asa SL, Kovacs K (1989) Effects of growth hormone-releasing hormone (GHRH) on densely granulated somatotroph adenomas and sparsely granulated somatotroph adenomas *in vitro*: a morphological and functional investigation. *J Endocrinol Invest* 12: 443-448

72. Kelijman M, Williams TC, Downs TR, Frohman LA (1988) Comparison of the sensitivity of growth hormone secretion to somatostatin *in vivo* and *in vitro* in acromegaly. *J Clin Endocrinol Metab* 67: 958-963

73. Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK, Low MJ (1997) Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. *Neuron* 19: 103-113

74. Koga M, Nakao H, Arao M, Sato B, Noma K, Morimoto Y, Kishimoto S, Mori S, Uozumi T (1987) Demonstration of specific dopamine receptors on human pituitary adenomas. *Acta Endocrinol (Copenh)* 114: 595-602

75. Kovacs K, Horvath E (1986) Tumors of the Pituitary Gland. *Atlas of Tumor Pathology*, Second Series, Fascicle 21. Armed Forces Institute of Pathology, Washington, D.C.

76. Kovacs K, Stefaneanu L, Ezzat S, Smyth HS (1994) Prolactin-producing pituitary adenoma in a male-to-female transsexual patient with protracted estrogen administration. A morphologic study. *Arch Pathol Lab Med* 118: 562-565

77. Krieger DT (1979) Medical treatment of Cushing disease. In: Tolis G, Labrie F, Martin JB, Naftolin F (eds) *Clinical Neuroendocrinology: A Pathophysiological Approach*. Raven Press, New York,423-427

78. Krieger DT (1983) Physiopathology of Cushing's disease. *Endocr Rev* 4: 22-43
79. Larson GH, Koos RD, Sortino MA, Wise PM (1990) Acute effect of basic fibroblast growth factor on secretion of prolactin as assessed by the reverse hemolytic plaque assay. *Endocrinology* 126: 927-932
80. Le Dafniet M, Blumberg-Tick J, Gozlan H, Barret A, Joubert Bression D, Peillon F (1989) Altered balance between thyrotropin-releasing hormone and dopamine in prolactinomas and other pituitary tumors compared to normal pituitaries. *J Clin Endocrinol Metab* 69: 267-271
81. Le Dafniet M, Blumberg-Tick J, Yuan Li J, Brandi AM, Bression D, Barret A, Feinstein MC, Peillon F (1988) Release of thyrotropin releasing hormone (TRH) from human prolactin-secreting pituitary adenoma cells. Modulation by dopamine [Fre]. *C R Acad Sci* [III] 306: 129-134
82. Le Dafniet M, Grouselle D, Li JY, Kujas M, Bression D, Barret A, Tixier-Vidal A, Peillon F (1987) Evidence of thyrotropin-releasing hormone (TRH) and TRH-binding sites in human nonsecreting pituitary adenomas. *J Clin Endocrinol Metab* 65: 1014-1019
83. Le Dafniet M, Lefebvre P, Barret A, Mechain C, Feinstein MC, Brandi AM, Peillon F (1990) Normal and adenomatous human pituitaries secrete thyrotropin-releasing hormone *in vitro*: modulation by dopamine, haloperidol, and somatostatin. *J Clin Endocrinol Metab* 71: 480-486
84. LeRiche V, Asa SL, Ezzat S (1996) Epidermal growth factor and its receptor (EGF-R) in human pituitary adenomas: EGF-R correlates with tumor aggressiveness. *J Clin Endocrinol Metab* 81: 656-662
85. Levy A, Lightman SL (1992) Growth hormone-releasing hormone transcripts in human pituitary adenomas. *J Clin Endocrinol Metab* 74: 1474-1476
86. Levy L, Bourdais J, Mouhieddine B, Benlot C, Villares S, Cohen P, Peillon F, Joubert D (1993) Presence and characterization of the somatostatin precursor in normal human pituitaries and in growth hormone secreting adenomas. *J Clin Endocrinol Metab* 76: 85-90
87. Li Y, Koga M, Kasayama S, Matsumoto K, Arita N, Hayakawa T, Sato B (1992) Identification and characterization of high molecular weight forms of basic fibroblast growth factor in human pituitary adenomas. *J Clin Endocrinol Metab* 75: 1436-1441
88. Lin S, Lin C, Gukovsky I, Lusis A, Sawchenko P, Rosenfeld MG (1993) Molecular basis of the little mouse phenotype and implications for cell type-specific growth. *Nature* 364: 208-213
89. Lloyd RV, Jin L, Chang A, Kulig E, Camper SA, Ross BD, Downs TR, Frohman LA (1992) Morphologic effects of hGRH gene expression on the pituitary, liver, and pancreas of MT-hGRH transgenic mice. An *in situ* hybridization analysis. *Am J Pathol* 141: 895-906
90. Loras B, Li JY, Durand A, Trouillas J, Sassolas G, Girod C (1985) GRF et adénomes somatotropes humains. Corrélations *in vivo* et *in vitro* entre la libération de GH et les aspects morphologiques et immunocytochimiques. *Ann Endocrinol (Paris)* 46: 373-382
91. Lüdecke DK, Westphal M, Schabet M, Höllt V (1980) *In vitro* secretion of ACTH, β-endorphin and β-lipotropin in Cushing's disease and Nelson's syndrome. *Horm Res* 13: 259-279
92. Marchionni MA, Goodearl ADJ, Chen MS, Bermingham-McDonogh O, Kirk C, Hendricks M, Danehy F, Misumi D, Sudhalter J, Kobayashi K, Wroblewski D, Lynch C, Baldassare M, Hiles I, Davis JB, Hsuan JJ, Totty NF, Otsu M, McBurney RN, Waterfield MD, Stroobant P, Gwynne D (1993) Glial growth factors are alternatively spliced erbB2 ligands expressed in the nervous system. *Nature* 362: 312-318
93. Mason IJ (1994) The ins and outs of fibroblast growth factors. *Cell* 78: 547-552
94. May V, Wilber JF, U'Prichard DC, Childs GV (1987) Persistence of immunoreactive TRH and GnRH in long-term primary anterior pituitary cultures. *Peptides* 8: 543-558
95. McAndrew J, Paterson AJ, Asa SL, McCarthy KJ, Kudlow JE (1995) Targeting of transforming growth factor-α expression to pituitary lactotrophs in transgenic mice results in selective lactotroph proliferation and adenomas. *Endocrinology* 136: 4479-4488
96. McCabe CJ, Gittoes NJ, Sheppard MC, Franklyn JA (1999) Thyroid receptor alpha1 and alpha2 mutations in nonfunctioning pituitary tumors. *J Clin Endocrinol Metab* 84: 649-653
97. McNicol AM, Kubba MAG, McTeague E (1988) The mitogenic effects of corticotrophin-releasing factor on the anterior pituitary gland of the rat. *J Endocrinol* 118: 237-241
98. Miller GM, Alexander JM, Bikkal HA, Katznelson L, Zervas NT, Klibanski A (1995) Somatostatin receptor subtype gene expression in pituitary adenomas. *J Clin Endocrinol Metab* 80: 1386-1392
99. Miller GM, Alexander JM, Klibanski A (1996) Gonadotropin-releasing hormone messenger RNA expression in gonadotropin tumors and normal human pituitary. *J Clin Endocrinol Metab* 81: 80-83
100. Missale C, Boroni F, Losa M, Giovanelli MA, Zanellato A, Dal Toso R, Balsari A, Spano P (1993) Nerve growth factor suppresses the transforming phenotype of human prolactinomas. *Proc Natl Acad Sci USA* 90: 7961-7965
101. Missale C, Boroni F, Sigala S, Zanellato A, Dal Toso R, Balsari A, Spano P (1994) Nerve growth factor directs differentiation of the bipotential cell line GH-3 into the mammotroph phenotype. *Endocrinology* 135: 290-298
102. Missale C, Losa M, Sigala S, Balsari A, Giovanelli M, Spano PF (1996) Nerve growth factor controls proliferation and progression of human prolactinoma cell lines through an autocrine mechanism. *Mol Endocrinol* 10: 272-285
103. Molitch ME (1987) Pathogenesis of pituitary tumors. *Endocrinol Metab Clin North Am* 16: 503-527
104. Murdoch GH, Potter E, Nicolaisen AK, Evans RM, Rosenfeld MG (1982) Epidermal growth factor rapidly stimulates prolactin gene transcription. *Nature* 300: 192-194

105. Nelson KG, Takahashi T, Lee DC, Luetteke NC, Bossert NL, Ross K, Eitzman BE, McLachlan JA (1992) Transforming growth factor-α is a potential medicator of estrogen action in the mouse uterus. *Endocrinology* 131: 1657-1664

106. Newman CB, Cosby H, Friesen HG, Feldman M, Cooper P, De Crescito V, Pilon M, Kleinberg DL (1987) Evidence for a nonprolactin, non-growth-hormone mammary mitogen in the human pituitary gland. *Proc Natl Acad Sci USA* 84: 8110-8114

107. Nicolis G, Shimshi M, Allen C, Halmi NS, Kourides IA (1988) Gonadotropin-producing pituitary adenoma in a man with long-standing primary hypogonadism. *J Clin Endocrinol Metab* 66: 237-241

108. Pagesy P, Yuan Li J, Berthet M, Peillon F (1992) Evidence of gonadotropin-releasing hormone mRNA in the rat anterior pituitary. *Mol Endocrinol* 6: 523-528

109. Patterson JC, Childs GV (1994) Nerve growth factor in the anterior pituitary: regulation of secretion. *Endocrinology* 135: 1697-1704

110. Peillon F, Le Dafniet M, Garnier P, Feinstein MC, Donnadieu M, Barret A, Gautron JP, Brandi AM, Benlot C, Lagoguey A, Lefebvre P, Blumberg-Tick J, Joubert (Bression) D (1989) Neurohormones coming from the normal and tumoral human anterior pituitary. Secretion and regulation *in vitro* [Fre]. *Pathologie Biologie* 37: 840-845

111. Peillon F, Liappi G, Garnier P, Brandi AM, Evain-Brion D, Dodeur M, Gautron JP, Donnadieu M, Michard M, Racadot J, Joubert (Bression) D (1988) *in vitro* secretion of somatostatin (SRIH) by human adenomatous somatotropic cells. Relation with somatotropic hormone (GH) release and modulation by thyroliberin (TRH) [Fre]. *C R Acad Sci* [III] 306: 161-166

112. Penabad JL, Bashey HM, Asa SL, Haddad G, Davis KD, Herbst AB, Gennarelli TA, Kaiser UB, Chin WW, Snyder PJ (1996) Decreased follistatin gene expression in gonadotroph adenomas. *J Clin Endocrinol Metab* 81: 3397-3403

113. Peters KG, Werner S, Chen G, Williams LT (1992) Two FGF receptor genes are differentially expressed in epithelial and mesenchymal tissues during limb formation and organogenesis in the mouse. *Develop* 114: 233-243

114. Plowman GD, Culouscou J-M, Whitney GS, Green JM, Carlton GW, Foy L, Neubauer MG, Shoyab M (1993) Ligand-specific activation of HER4/p180^{erbB4}, a fourth member of the epidermal growth factor receptor family. *Proc Natl Acad Sci USA* 90: 1746-1750

115. Polk DH, Ervin MG, Padbury JF, Lam RW, Reviczky AL, Fisher DA (1987) Epidermal growth factor acts as a corticotropin-releasing factor in chronically catheterized fetal lambs. *J Clin Invest* 79: 984-988

116. Prysor-Jones RA, Silverlight JJ, Jenkins JS (1989) Oestradiol, vasoactive intestinal peptide and fibroblast growth factor in the growth of human pituitary tumour cells *in vitro*. *J Endocrinol* 120: 171-177

117. Qian X, LeVea CM, Freeman JK, Dougall WC, Greene MI (1994) Heterodimerization of epidermal growth factor receptor and wild-type or kinase-deficient Neu: A mechanism of interreceptor kinase activation and transphosphorylation. *Proc Natl Acad Sci USA* 91: 1500-1504

118. Ramsdell JS, Tashjian AH (1985) Thyrotropin-releasing hormone and epidermal growth factor stimulate prolactin synthesis by a pathway(s) that differs from that used by phorbol esters: dissociation of actions by calcium dependency and additivity. *Endocrinology* 117: 2050-2060

119. Ray DW, Littlewood AC, Clark AJ, Davis JRE, White A (1994) Human small cell lung cancer cell lines expressing the proopiomelanocortin gene have aberrant glucocorticoid receptor function. *J Clin Invest* 93: 1625-1630

120. Reubi JC, Landolt AM (1989) The growth hormone responses to octreotide in acromegaly correlate with adenoma somatostatin receptor status. *J Clin Endocrinol Metab* 68: 844-850

121. Rizzino A (1991) Growth Factors. In: Kovacs K, Asa SL (eds) *Functional Endocrine Pathology*. Blackwell Scientific Publications Inc., Boston, 979-989

122. Roh M, Paterson AJ, Asa SL, Chin E, Kudlow JE (2001) Stage-sensitive blockade of pituitary somatomammotrope development by targeted expression of a dominant negative epidermal growth factor receptor in transgenic mice. *Mol Endocrinol* 15: 600-613

123. Saeger W (1977) Die Morphologie der paraadenomatösen Adenohypophyse. Ein Beitrag zur Pathogenese der Hypophysenadenome. *Virchows Arch [Pathol Anat]* 372: 299-314

124. Sakai Y, Horiba N, Sakai K, Tozawa F, Kuwayama A, Demura H, Suda T (1997) Corticotropin-releasing factor up-regulates its own receptor gene expression in corticotropic adenoma cells *in vitro*. *J Clin Endocrinol Metab* 82: 1229-1234

125. Samsoondar J, Kudlow JE (1987) Partial purification of an adrenal growth factor produced by normal bovine anterior pituitary cells in culture. *Endocrinology* 120: 929-935

126. Sanno N, Jin L, Qian X, Osamura RY, Scheithauer BW, Kovacs K, Lloyd RV (1997) Gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor messenger ribonucleic acids expression in nontumorous and neoplastic pituitaries. *J Clin Endocrinol Metab* 82: 1974-1982

127. Sano T, Asa SL, Kovacs K (1988) Growth hormone-releasing hormone-producing tumors: clinical, biochemical, and morphological manifestations. *Endocr Rev* 9: 357-373

128. Schechter J, Goldsmith P, Wilson C, Weiner R (1988) Morphological evidence for the presence of arteries in human prolactinomas. *J Clin Endocrinol Metab* 67: 713-719

129. Scheithauer BW, Kovacs K, Randall RV (1983) The pituitary gland in untreated Addison's disease. A histologic and immunocytologic study of 18 adenohypophyses. *Arch Pathol Lab Med* 107: 484-487

130. Scheithauer BW, Kovacs K, Randall RV, Ryan N (1985) Pituitary gland in hypothyroidism. Histologic and immunocytologic study. *Arch Pathol Lab Med* 109: 499-504

131. Scheithauer BW, Sano T, Kovacs KT, Young WF, Jr., Ryan N, Randall RV (1990) The pituitary gland in pregnancy: A clinicopathologic and immunohistochemical study of 69 cases. *Mayo Clin Proc* 65: 461-474

132. Senogles SE, Benovic JL, Amlaiky N, Unson C, Milligan G, Vinitsky R, Spiegel AM, Caron MG (1987) The D2 receptor of anterior pituitary is functionally associated with a pertussis toxin-sensitive guanine nucleotide binding protein. *J Biol Chem* 262: 4860-4867

133. Shimon I, Hüttner A, Said J, Spirna OM, Melmed S (1996) Heparin-binding secretory transforming gene (*hst*) facilitates rat lactotrope cell tumorigenesis and induces prolactin gene transcription. *J Clin Invest* 97: 187-195

134. Snyder PJ (1985) Gonadotroph cell adenomas of the pituitary. *Endocr Rev* 6: 552-563

135. Spada A, Bassetti M, Martino E, Giannattasio G, Beck-Peccoz P, Sartorio A, Vallar L, Baschieri L, Pinchera A, Faglia G (1985) *In vitro* studies on TSH secretion and adenylate cyclase activity in a human TSH-secreting pituitary adenoma. Effects of somatostatin and dopamine. *J Endocrinol Invest* 8: 193-198

136. Spada A, Elahi FR, Arosio M, Sartorio A, Guglielmo L, Vallar L, Faglia G (1987) Lack of desensitization of adenomatous somatotrophs to growth hormone-releasing hormone in acromegaly. *J Clin Endocrinol Metab* 64: 585-591

137. Spada A, Lania A (1996) Hormone receptors in pituitary adenomas. In: Landolt AM, Vance ML, Reilly PL (eds) *Pituitary adenomas*.Churchill Livingstone, New York,59-71

138. Suda T (1992) Corticotropin-releasing factor gene expression. *Methods in Neuroscience* 9: 23-31

139. Suda T, Tozawa F, Dobashi I, Horiba N, Ohmori N, Yamakado M, Yamada M, Demura H (1993) Corticotropin-releasing hormone, proopiomelaninocortin, and glucocorticoid receptor gene expression in adrenocorticotropin-producing tumors *in vitro*. *J Clin Invest* 92: 2790-2795

140. Suda T, Tozawa F, Yamada M, Ushiyama T, Tomori N, Sumitomo T, Nakagami Y, Demura H, Shizume K (1988) Effects of corticotropin-releasing hormone and dexamethasone on proopiomelanocortin messenger RNA level in human corticotroph adenoma cells *in vitro*. *J Clin Invest* 82: 110-114

141. Thapar K, Kovacs K, Stefaneanu L, Scheithauer B, Killinger D, Lloyd RV, Smyth HS, Barr A, Thorner MO, Gaylinn B, Laws ER, Jr. (1997) Overexpression of the growth-hormone-releasing hormone gene in acromegaly-associated pituitary tumors. An event associated with neoplastic progression and aggressive behavior. *Am J Pathol* 151: 769-784

142. Thorner MO, Perryman RL, Cronin MJ, Rogol AD, Draznin M, Johanson A, Vale W, Horvath E, Kovacs K (1982) Somatotroph hyperplasia: Successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. *J Clin Invest* 70: 965-977

143. Vallar L, Meldolesi J (1989) Mechanisms of signal transduction at the dopamine D2 receptor. *Trends in Pharmacological Science* 10: 74-77

144. Wang CJ, Howng SL, Lin KH (1995) Expression of thyroid hormone receptors in human pituitary tumor cells. *Cancer Lett* 91: 79-83

145. Webster J, Ham J, Bevan JS, Scanlon MF (1989) Growth factors and pituitary tumors. *Trends Endocrinol Metab* 1: 95-98

146. Webster J, Ham J, Bevan JS, ten Horn CD, Scanlon MF (1991) Preliminary characterization of growth factors secreted by human pituitary tumors. *J Clin Endocrinol Metab* 72: 687-692

147. Weiner RI, Windle J, Mellon P, Schechter J (1991) Role of FGF in tumorigenesis of the anterior pituitary. *J Endocrinal Invest* 14 (Suppl): S13

148. Werner S, Weinberg W, Liao X, Peters KG, Blessing M, Yuspa SH, Weiner RL, Williams LT (1993) Targeted expression of a dominant-negative FGF receptor mutant in the epidermis of transgenic mice reveals a role of FGF in keratinocyte organization and differentiation. *EMBO J* 12: 2635-2643

149. White BA, Bancroft FC (1983) Epidermal growth factor and thyrotropin-releasing hormone interact synergistically with calcium to regulate prolactin mRNA levels. *J Biol Chem* 258: 4618-4622

150. White MC, Daniels M, Kendall-Taylor P, Turner SJ, Mathias D, Teasdale G (1985) Effects of growth hormone-releasing factor (1-44) on growth hormone release from human somatotrophinomas *in vitro*: interaction with somatostatin, dopamine, vasoactive intestinal peptide and cycloheximide. *J Endocrinol* 105: 269-276

151. Wood DF, Johnston JM, Johnston DG (1991) Dopamine, the dopamine D2 receptor and pituitary tumours. *Clin Endocrinol (Oxf)* 35: 455-466

152. Yamada M, Hashimoto K, Satoh T, Shibusawa N, Kohga H, Ozawa Y, Yamada S, Mori M (1997) A novel transcript for the thyrotropin-releasing hormone receptor in human pituitary and pituitary tumors. *J Clin Endocrinol Metab* 82: 4224-4228

153. Yamada M, Monden T, Satoh T, Sato N, Murakami M, Iriuchijima T, Kakegawa T, Mori M (1993) Pituitary adenomas of patients with acromegaly express thyrotropin-releasing hormone receptor messenger RNA cloning and functional expression of the human thyrotropin-releasing hormone receptor gene. *Biochem Biophys Res Commun* 195: 737-745

154. Yan G, Fukabori Y, McBride G, Nikolaropolous S, McKeehan WL (1993) Exon switching and activation of stromal and embryonic fibroblast growth factor (FGF)-FGF receptor genes in prostate epithelial cells accompany stromal independence and malignancy. *Mol Cell Biol* 13: 4513-4522

155. Yan G, Wang F, Fukabori Y, Sussman D, Hou J, McKeehan WL (1992) Expression and transformation of a variant of the heparin-binding fibroblast growth factor receptor (*flg*) gene resulting from splicing of the exon at alternate 3′-acceptor site. *Biochem Biophys Res Commun* 183: 423-430

156. Ying S-Y (1988) Inhibins, activins, and follistatins: gonadal proteins modulating the secretion of follicle-stimulating hormone. *Endocr Rev* 9: 267-293

157. Zimering MB, Katsumata N, Sato Y, Brandi ML, Aurbach GD, Marx SJ, Friesen HG (1993) Increased basic fibroblast growth factor in plasma from multiple endocrine neoplasia type 1: Relation to pituitary tumor. *J Clin Endocrinol Metab* 76: 1182-1187