Review

Biomarkers of Pituitary Neoplasms

AYDIN SAV[1], FABIO ROTONDO[2], LUIS V. SYRO[3], BERND W. SCHEITHAUER[4§] and KALMAN KOVACS[2]

[1]*Department of Pathology, Acıbadem University, School of Medicine, Maltepe, Istanbul, Turkey;*
[2]*Department of Laboratory Medicine, St Michael's Hospital, University of Toronto, Toronto, ON, Canada;*
[3]*Department of Neurosurgery, Pablo Tobon Uribe Hospital and Clinica Medellin, Medellin, Colombia;*
[4]*Department of Anatomic Pathology, Mayo Clinic, Rochester, MN, U.S.A.*

Abstract. *In a wide spectrum of tumors, cell proliferation, vascularity, apoptosis, cell adhesion, and cell-cycle progression may indicate tumor progression. In this review article, the literature regarding apoptotic markers and p53, as well as cyclooxygenase-2, galectin-3, and pituitary tumor-transforming factor, proliferative markers, angiogenesis, including vascular endothelial growth factor and its receptor, pituitary tumor-transforming gene, microarrays, stem cells, and microenvironment and tumor heterogeneity are presented. Only a particular group of selected biomarkers show promise in differentiating pituitary tumors which will behave in an aggressive manner. Therefore, the most common and promising biomarkers and terms were analyzed, proposing the need for uniform design and application of methods and standardized criteria for the interpretation of results. The new spectrum of biomarkers may shed light upon the pathogenetic mechanisms and also may serve as standardized diagnostic tool for daily pathologic practice.*

A number of biomarkers are documented to be of predictive value with regard to clinical and radiological criteria for the management of unpredictably behaving pituitary tumors. Among these are the apoptotic index, inhibitory cell cycle proteins, matrix metalloproteinases (MMPs) (1), p53 and p21, and markers of angiogenesis, namely vascular endothelial growth factor (VEGF) (2, 3-6) and proliferative markers, *e.g.* Ki-67, and topoisomerases. Newer markers such as cyclooxygenase-2 (COX-2) and galectin-3 have been added to the list (4, 7-11). Initial results of the studies using these other markers have also been inconsistent. The published inconsistencies may in part be attributed to variations in the definition of invasion. It is fascinating that in some studies, presence or absence of tumor invasion is solely based on preoperative radiological and/or intraoperative surgical findings, whereas others define it as a histologically-proven observation. Yet another factor may be the inclusion of a variety of pituitary subtypes instead of single or distinct pathological tumor types. Some markers are restricted to specific cell types, such as galectin-3, which is expressed by prolactin (PRL) and corticotropin (ACTH) -secreting cells, but not by most other cell types. (8) Even though some of the emerging biomarkers show promise as having predictive value in pituitary tumors, the need for uniform inclusion criteria and study design is discussed. Herein, a limited spectrum of markers of pituitary adenomas, with respect to their strength as predictors of tumor behavior is discussed.

Apoptosis

Apoptosis, or programmed cell death, is characterized by a rapid sequence of events leading to the elimination of damaged cells (12). It is defined by morphological changes including cellular shrinkage and nuclear demarcation. In neoplasms, due to the disturbance of the normal balance between mitotic and apoptotic activity which contributes to increased tumor growth, apoptosis is generally suppressed. Studies of apoptosis in pituitary tumors are limited in number. Vidal *et al.* (13), examining more than 8,000 pituitary tumor biopsy specimens, reported that most apoptotic activity was observed in corticotroph adenomas,

§Died on September 19, 2011.

Correspondence to: Aydin Sav, MD, Acibadem University, School of Medicine, Department of Pathology, Gulsuyu Mahallesi, Fevzi Çakmak Cd, Divanyolu Sok, 1 Maltepe, Istanbul, Turkey. Tel: 90 2165443762, Fax: 90 2165775357, e-mail: murataydinsav@gmail.com

Key Words: Angiogenesis, apoptosis, p53, p27, cox-2, folliculostellate cells, galectin-3, hif-1alpha, inflammation and tumor, matrix metalloproteinases (MMPS), microarray, microenvironment, microvessel density (MVD), pituitary stem cells, proliferative marker (ki-67), pituitary tumor-transforming gene (PTTG), topoisomerase-2-alpha, tumor heterogeneity, VEGF, review.

with only occasional examples being seen in PRL- or gonadotropin secreting adenomas. Several studies investigated the relevance of apoptotic activity as a clinicopathological marker (14, 15). Kontogeorgos *et al*. (16) noted higher apoptotic activity in aggressive, drug-resistant adenomas, indicating that apoptosis may be a useful prognostic marker. Kulig *et al*. (15) reported similar results, with a four-fold increase in apoptotic activity in pituitary carcinomas compared with adenomas. Likewise, lower expression of BCL2 antiapoptotic factor was seen in pituitary carcinomas compared with adenomas and the non-tumoral pituitary gland (15). In contrast, Ibrahim *et al*. (14) found that apoptotic indices were not predictive of the growth rate of non-functioning pituitary tumors. These findings were consistent with those of Nakabayashi *et al*. (17), who found no significant difference between apoptotic indices in recurring and non-recurring adenomas. Last of all, Losa *et al*. (18), in their relatively coherent series, found no significant difference in apoptotic index between Adrenocorticotrophic hormone (ACTH)-expressing macro- and microadenomas. With respect to apoptotic index and its correlation with clinicopathological parameters, results are highly variable and fail to support its utility as a prognostic indicator (61, 64-68). Apparently, Kontogoergos *et al*. (16) showed that hormone-secreting adenomas had higher indices than did non-functioning tumors; highest apoptotic indices were observed in thyroid-stimulating hormone (TSH)-secreting adenomas, followed by growth hormone (GH)-, prolactin (PRL) - and mixed GH/PRL-secreting adenomas. Correspondingly, Sambaziotis *et al*. (19) found that functioning adenomas exhibit higher apoptotic indices than non-functioning ones. On the contrary, another study showed a higher apoptotic index in non-functioning tumors compared with GH-secreting adenomas (33% *versus* 11%), a difference not statistically significant (20). The expression of BCL2 and BAX, anti-apoptotic and proapoptotic factors, respectively, also correlates with apoptotic indices. The BCL2/BAX ratio has been found to be higher in non-functioning adenomas, highlighting the differences in the balance of anti- to proapoptotic activity in these tumors (19). Although these findings suggest that the BCL2/BAX ratio is a useful marker of apoptosis and possibly of tumor behavior, Ozer *et al*. found decreased expression of BCL2 in non-functioning as well as in PRL -secreting adenomas (21). It was concluded that down-regulation of the BAX protein was associated with pituitary tumor progression (21). These discrepant findings may be attributed to the evaluation of BCL2 and BAX expression rather than the BCL2/BAX ratio, which might be a better prognostic indicator. Similarly, although elevated p53 expression levels are shown to correlate with aggressive tumor behavior, not all studies confirm these findings. (5, 21-25) Therefore, the relevance of the apoptotic index and p53 expression to tumor behavior is debatable.

p53

Until recently, apoptosis was not recognized by the use of routine histological stains. Various descriptive terms corresponding to the apoptotic events are found, even in classic textbooks of pathology: 'karyopyknosis'; 'karyorhexis' and 'karyolysis' referring to nuclear changes; 'hyaline bodies', 'Camino bodies', and 'Shivata bodies' describe cytoplasmic remnants; 'cannibalism', 'cell-in-cell' and 'tiger-eye' are used to illustrate the phagocytotic events of late apoptotic phases; and lastly, 'thanatosomes' refer to apoptotic cell remnants. All these terms should be abandoned and substituted by the term 'apoptotic figures' (26). Electron microscopy can demonstrate apoptotic cells with accuracy. However, due to limited numbers of adenoma cells included in tissue samples, isolation of apoptotic cells is difficult or inexplorable. The spectrum of apoptosis in pituitary adenomas has recently been described in detail (13). Expression of *p53* gene products is important for tumor biology. Although *p53* mutations has not been documented in pituitary adenomas, *p53* immunoreactivity have been found to correlate with tumor invasiveness (11, 26). p53 expression has been linked to aggressive tumor behavior (11, 26).

Pituitary adenomas are neoplasms with a low proliferation rate, and thus, like mitoses, apoptosis is absent or difficult to identify in routine stains (26). Thapar *et al*. (27) demonstrated a significant association ($p<0.001$) between tumor behavior and p53 expression, labeling of 0%, 15.2%, and 100% being seen in non-invasive and invasive adenomas and carcinomas, respectively, Wierinckx *et al*. reported significantly higher p53 expression in 'aggressive-invasive' tumors compared with those with less aggressive behavior ($p=0.0001$) (11). Ozer *et al*. showed that elevated p53 expression was an independent indicator of local relapse (21), suggesting that p53 status is associated with tumor progression. In contrast to these reports, other studies of p53 levels and tumor recurrence, invasiveness, and/or volume found no correlations. Therefore, it is questionable in regard to the relevance of p53 expression as a marker of recurrence (5, 23, 24). Although there is an emerging significance of p53/p21-dependent senescence pathways, this issue is not yet clarified (28, 29). Therefore, additional research is needed to clarify the association between p53 expression and pituitary tumor behavior.

p27

p27 KIP1, a cyclin-dependent kinase inhibitor, is involved in regulation of cell-cycle progression (2, 30, 31). The $p27^{KIP1}$ gene has a DNA sequence similar to other members of the 'CIP/KIP' family which include the $p21^{CIP1/WAF1}$ and $p57^{KIP2}$ genes. Moreover, due to this structural similarity the 'CIP/KIP' proteins share the functional characteristic of

being able to bind several different classes of cyclin and CDK molecules. For example, $p27^{KIP}$ binds to cyclin-D either alone, or when complexed to its catalytic subunit CDK4. In doing so, $p27^{KIP1}$ inhibits the catalytic activity of CDK4, which means that it prevents CDK4 from adding phosphate residues to its principal substrate, the retinoblastoma (pRb) protein. Increased levels of $p27^{KIP1}$ protein typically cause cells to arrest in the G_1 phase of the cell cycle. Likewise, $p27^{KIP1}$ is able to bind other CDK proteins when complexed to cyclin subunits, such as cyclin E/CDK2 and cyclin A/CDK2.

In most cases, extracellular growth factors which prevent cell growth cause an increase in $p27^{KIP1}$ levels inside a cell. For example, levels of $p27^{KIP1}$ increase when transforming growth factor (TGF) is present outside of epithelial cells, causing growth arrest (32). In contrast, interleukin (IL-2) causes $p27^{KIP1}$ levels to decrease in T-lymphocytes. A mutation in the IL-2 gene may lead to loss of control over the cell cycle leading to uncontrolled cellular proliferation (33-35). Loss of p27 expression has been observed in metastatic canine mammary carcinomas (36, 37). Decreased TGF-beta signaling has been suggested to cause loss of p27 expression in this tumor type (36).

In the earliest study of p27 KIP1 in human pituitaries, Lloyd *et al*. found decreased p27 KIP1 expression in pituitary neoplasms compared with the normal gland (30, 31). Inevitably, p27 KIP1 immunoexpression was inversely-correlated with the staining for the proliferation marker Ki-67, suggesting that p27 KIP1 is an additional predictive marker of pituitary tumor behavior (30, 31). In the same way, several studies found significantly lower p27 KIP1 levels in non-functioning adenomas and other studies found higher proliferation rates in these tumors (23, 38). In keeping with these findings, Scheithauer *et al*. found that p27 KIP1 expression was lower in carcinomas compared with invasive adenomas (23). Nakabayashi *et al*. found that p27 KIP1 expression was lower in recurrent adenomas compared with non-recurrent ones (17). These results always show reduced expression of p27 KIP1 and p21 in pituitary adenomas and malignant tumors. However, investigation of aberrations in *p21* and *p27 KIP1* genes showed no mutations in one study (39) and in another study, no differences in p27 KIP1 protein levels were detected in 18 pituitary tumors, five of which actually had a polymorphism of p27 KIP1 (codon 109, Val→Gly) (40). Correspondingly, Jin *et al*. found no differences in *p27 KIP1* mRNA expression among non-tumorous, adenomatous, and metastatic pituitary tumors (8). Thus, the lower expression of these cell-cycle inhibitors may be caused by post-translational mechanisms, such as increased degradation (41).

Investigating the expression of phosphorylated p27 KIP1, the inactivated form of p27 KIP1, Korbonits *et al*. found that corticotrophin-secreting adenomas exhibited higher levels than other adenomas (41). The latter exhibited reduced phosphorylated p27 KIP1 compared with normal pituitary, whereas its levels were similar in normal pituitary and corticotrophin-secreting tumors (41). The ratio of phosphorylated p27 KIP1 to p27 KIP1 was significantly higher in corticotroph-secreting adenomas compared with metastatic tumors, invasive tumors, and TSH-secreting adenomas (41). In pituitary carcinomas, both phosphorylated p27 KIP1 and p27 KIP1 levels were decreased. The variable ratio of phosphorylated p27 KIP1 to p27 KIP1 in pituitary tumors may show that the balance between the phosphorylated and unphosphorylated forms of p27 KIP1 protein may regulate tumor progression (41). Earlier studies demonstrated reduced p27 KIP1 levels in ACTH-secreting adenomas (2, 8, 38). Luteinizing hormone-expressing and TSH-secreting adenomas stained more frequently for p27 KIP1; ACTH-secreting adenomas had the lowest levels of p27 KIP1 protein in the study of Jin *et al*. (8). Similarly, Bamberger *et al*. showed that p27 KIP1 negative cells occurred more often in corticotrophin-secreting adenomas in contrast to gonadotropin-secreting adenomas, in which p27 KIP1 expression was higher than in other pituitary adenoma subtypes (2). The significance and mechanisms underlying reduced p27 KIP1 levels in pituitary tumors is uncertain. Whether reduced p27 KIP1 and/or p21 expression is a primary event in pituitary tumor initiation and progression or is secondary to other tumorigenic factors is unclear and requires further analysis.

Topoisomerase 2 Alpha

Topoisomerases (type I and type II) are enzymes that wind and unwind DNA, in order for DNA to control the synthesis of proteins, and to facilitate DNA replication. The structure of DNA is a double-stranded helix, wherein the four bases, adenine, thymine, guanine, and cytosine, are paired and stored in the center of this helix. While this structure provides a stable means of storing the genetic code, Watson and Crick noted that the two strands of DNA are intertwined, and this would require the two strands to be untwisted in order to access the stored information. They also foresaw that there would be some mechanism to overcome this problem (12). Type II topoisomerase cuts both strands of one DNA double helix, passes another unbroken DNA helix through it, and then re-anneals the cut strand. There are two subclasses of this enzyme: type IIA and type IIB topoisomerases, which share a similar structure and mechanisms. Examples of type IIA topoisomerases include eukaryotic topo-II, *Escherichia coli* gyrase, and *E. coli* topo IV. Examples of type IIB topoisomerase include topo VI. Many drugs operate through interference with the topoisomerases (42). The broad-spectrum fluoroquinolone antibiotics act by disrupting the function of bacterial type II topoisomerases. These small-

molecule inhibitors act as efficient anti-bacterial compounds by exploiting the natural ability of topoisomerase to create breaks in chromosomal DNA. Topoisomerase inhibitors are used in chemotherapy work by interfering with mammalian-type eukaryotic topoisomerases in cancer cells. This induced breaks in DNA that ultimately direct cells to programmed cell death, *i.e.* apoptosis. This DNA-damaging effect, as well as having curative properties, may lead to secondary neoplasms in treated persons.

Several molecules are involved in the regulation of the cell cycle. Topoisomerase II alpha (TOPOIIA) is a key enzyme involved in DNA replication, cell-cycle progression and chromosome segregation, which peaks through the G_2 to the M phase of the cell cycle, minimized at the end of mitosis and is not found in resting cells. TOPOIIA expression correlates with cell proliferation, and thus, it has been suggested as a proliferation marker (43). TOPOIIA protein also represents a molecular target for several inhibitors, including doxorubicin, which is important for the treatment of some topoisomerases (44, 45). The value of TOPOIIA in pituitary adenomas is low, for the reported results are similar to that for Ki-67 (46, 47). The question of whether TOPOIIA is a valuable biomarker of tumor aggressiveness needs further studies.

Cyclooxygenase-2 (COX-2)

COX-2, a key enzyme mediating prostaglandin synthesis, is not only involved in inflammatory responses, but is implicated in tumor invasiveness and angiogenesis (9). Its expression in pituitary tumors was recently demonstrated. Increased COX-2 expression was particularly evident in pituitary carcinomas compared with adenomas and normal pituitary, hence suggesting an important role in tumor progression. Onguru *et al*. found increased COX-2 expression in functioning *versus* non-functioning tumors, both of which had lower levels of COX-2 than did carcinomas (48). Bloomer *et al*. found COX-2 expression in 83% of pituitary tumors (n=30) (7). Its expression was significantly associated with that of luteinizing hormone and TSH (7). On the contrary, in a larger series of 164 pituitary tumors, Vidal *et al*. (49) found GH, PRL, TSH, and female gonadotrophs to express lower COX-2 levels than male gonadotrophs and oncocytic and nononcocytic null-cell adenomas. Analyses should be focused on several clinical variables, including sex, in gonadotrophic tumors because compared with other pituitary neoplasms, they express higher levels of COX-2 (7, 49). There is also a significant association between COX-2 expression and patient age; no correlations were noted with patient sex or with tumor size and invasiveness (49). COX-2 expression did, however, show a strong correlation with Microvessel density (MVD). The role of COX-2 in pituitary malignant transformation requires further research because COX-2 is significantly highly-expressed in pituitary carcinomas compared with adenomas.

Galectin-3

Galectin-3 has been implicated in several biological processes, including tumor progression, apoptosis, and metastasis (50). In a study of 162 pituitary tumors, including 14 carcinomas, Riss *et al*. found galectin-3 to be expressed only in PRL- and ACTH-secreting tumors, with all other tumor types being immunonegative (50). Moreover, galectin-3 staining was found to be significantly higher in ACTH-secreting carcinomas compared with adenomas (50). This specific pattern of galectin-3 expression by PRL- and ACTH-secreting tumors may be significant because many pituitary carcinomas are PRL- or ACTH-producing carcinomas. Additionally, higher galectin-3 expression was found in functioning ACTH-secreting adenomas compared with silent corticotrophin-secreting adenomas (8, 10). As galectin-3 appears to be a promising marker, validation studies to crystallize its role as a marker of pituitary tumor behavior are warranted. Studies of galectin-3 expression are limited in number, but show promise (8, 10, 50). In as much as galectin-3 is exclusively expressed in ACTH- and PRL-secreting tumors, the most common form of pituitary carcinoma, further studies may establish a role for galectin-3 in tumor differentiation and aggressiveness.

Pituitary Tumor-transforming Gene (PTTG)

PTTG was isolated from experimental rat pituitary tumors (51). PTTG expression is induced by estrogens and it stimulates basic fibroblast growth factor (bFGF) production (52). The human homologs of PTTG comprise of several separate genes. PTTG is located on chromosome 5q33 and is abundantly expressed in most human pituitary tumors (53), as well as non-pituitary neoplasms. The role of PTTG in the early transformation of pituitary cells through hyperplasia to frank adenoma formation has been demonstrated in experimental rat prolactinomas (52).

Subsequent studies have identified PTTG as the human homolog of securin, a protein mediating sister chromatid separation during mitosis (54). Nonetheless, its expression in pituitary tumors has been demonstrated by many studies (55-60). Recent studies support the role of PTTG in p21-mediated senescence, in accordance with the predominantly benign phenotype of pituitary neoplasms. When *PTTG* mRNA expression was compared in 54 pituitary tumors, Zhang *et al*. found no correlation with radiological tumor stage in clinically non-functioning adenomas, but did note significantly higher levels in hormone-secreting invasive tumors compared with non-invasive ones (53). These results suggested different mechanisms of PTTG action and/or expression in these two groups. Additionally, Hunter *et al*. showed that *PTTG* mRNA levels were higher in GH-secreting adenomas than in non-functioning tumors, with an increase

of 2.7-fold (59). Although PTTG levels were higher in these tumors compared with PRL- and ACTH-secreting adenomas, no statistical significance was found (59). Several studies also investigated correlations between the expression of PTTG and other markers relevant to pituitary tumors, particularly VEGF and bFGF, because PTTG promotes angiogenesis in many settings (55, 56, 61). A study of 103 pituitary adenomas showed a significant positive correlation between *PTTG* and *VEGF* mRNA levels, as well as between PTTG and VEGF receptor (KDR) expression levels (55). Yet another study found many tumor cells to exhibit colocalization of PTTG with VEGF, as well as a high correlation between PTTG expression and the number of CD34-blood vessels in GH-secreting pituitary adenomas (61). These results suggest a promising role for PTTG in the regulation of pituitary angiogenesis. Studying the utility of PTTG in distinguishing between recurrent and non-recurrent tumors, Filippella *et al*. found a cutoff value of 3.3%, with 60% sensitivity and 76% specificity (58). Nevertheless, there was no significant correlation between PTTG immunopositivity and tumor size or grade, patient age or sex, or tumor treatment (58). Further studies are required to determine the importance of PTTG in pituitary tumor development.

Recent studies support the role of emerging technologies in finding new markers of the biology and behavior of pituitary adenomas. Ruebel *et al*., by using microarray analysis, demonstrated differential gene expression profiles for various pituitary adenoma subtypes and uncovered novel genes of possible value as predictive markers (62). Using microarray technology to analyze PRL-expressing tumors, Wierinckx *et al*. found a set of diagnostic markers that included PTTG (11). The complexity of interactions between several molecular and cellular pathways in pituitary tumor development and progression may account for the current lack of success in the search for a single predictive marker. Emerging techniques make feasible the identification and validation of a prognostic marker set that may aid the clinician in predicting tumor behavior. The most conclusive observation, with respect to PTTG, appears to be its up-regulation of VEGF and FGF expression, both these being overexpressed in various malignancies (55, 56, 61). Further studies into the role and prognostic value of PTTG in pituitary tumors are necessary. It will also be important to investigate the PTTG and p21 levels and the potential association between these molecules in various pituitary adenoma subtypes in light of recent findings of PTTG-induced p21-mediated premature senescence in mouse models (28, 29).

Proliferative Marker Ki-67

Ki-67 has been widely used as an immunohistochemical marker in the study of pituitary tumors; many studies investigated its potential links to other, newer biomarkers. Several studies suggest that Ki-67 values greater than 3% predict more aggressive tumor behavior; on the other hand, no firm consensus has been reached regarding a precise cutoff value so far (63). The issue is confounded by considerable overlap of Ki-67 between indolent and aggressive adenomas. Therefore, the predictive value of this marker remains an issue, with respect to aggressive behavior. Similarly, whereas it is generally believed that a high Ki-67 labelling index (LI) is a predictor of recurrence, there are numerous reports of high Ki-67 LIs in non-recurring tumors. Review of the current literature reveals that the very definition of recurrence affects the outcome of studies investigating a correlation with Ki-67 labeling (63).

To summarize, using more uniform criteria will, with no doubt, yield more dependable results. There are consistent findings of several studies with uniform criteria for recurrence that successfully correlate Ki-67 LI with tumor regrowth. Although the Ki-67 LI-alone at times fails as a predictor of recurrence, it may prove useful when combined with information regarding tumor behavior or with other biomarkers. Ki-67 labeling and tumor size, when extracted from data regarding function, are interrelated, partly due to the slowly growing nature of most pituitary adenomas. Slowly growing non-functioning pituitary tumors which might become symptomatic when sufficiently voluminous often have very low Ki-67 LIs, therefore skewing the association of tumor size with Ki-67 labeling. Likewise, tumor subtype, growth rate, and the Ki-67 LI may show wide fluctuations (64). In order to propose a reproducible numeric spectrum for pituitary tumours, criteria should be agreed which will enhance the consistency of findings.

Neither clinicians nor neurosurgeons can rely on the Ki-67 LI as a prognostic marker; therefore this emphasizes the urgent need for new markers. Emerging studies using deoxyribonucleic acid and microribonucleic acid microarray analyses show much promise in identifying valuable prognostic markers (62, 65, 66). In fact, recent microarray studies showed that using a set of markers is superior to the use of single markers as prognostic indicators of tumor behavior, particularly for aggressiveness (11). Battoni *et al*. studied the entire microribonucleic acid content of pituitary adenomas and revealed a set of 29 differentially expressed microribonucleic acids predictive of tumor histotype, size, and drug treatment (65). Interestingly, several of these were implicated in cell proliferation and apoptosis. Microarray studies have also identified differential expression of genes, thus far, not investigated as prognostic indicators (62). Emerging data will, under no doubt, aid researchers in gaining a deeper understanding of pituitary tumor development and progression. Critically assessed, the results of such studies will certainly aid clinicians in the management of aggressive pituitary tumors (63).

Angiogenesis

Angiogenesis is defined as the formation of new blood vessels and is associated with tumor progression (13). It is axiomatic that tumor growth requires neovascularization to supply tumor cells with the necessary nutrients and oxygen. Therefore, tumor vascularity is often associated with tumor growth, aggressive behavior, and metastatic potential (13). The utility of markers of angiogenesis as prognostic indicators of pituitary tumors is addressed by several studies investigating expression of VEGF, endothelial growth factor, COX-2, and hypoxia-inducible factor-1a (HIF-1a).

VEGF. VEGF is an important angiogenic factor that mediates endothelial cell proliferation, vascular permeability and cell motility. Its expression is related to tumor angiogenesis and often to aggressive behavior (67). Findings of VEGF correlations with tumor invasiveness and proliferation are inconsistent, indicating that VEGF may not directly contribute to tumoral invasion, but may regulate pathways that increase tumor volume or mediate invasiveness (4, 55, 68-72). This notion is supported by the observation that VEGF expression is not strictly associated with endothelium and vessels, but also occurs in adenoma cells as well (4, 67). Expression of VEGF receptors in pituitary adenomas has also been investigated. Higher expression of fetal liver kinase-1, a form of VEGF receptor that mediates mitogenesis and affects endothelial cell morphology, was associated with extracellular extension (55). Furthermore, fetal liver kinase-1 expression was significantly higher in non-functioning compared with functioning tumors (55). Other studies regarding the expression of VEGF and its receptors in the various pituitary adenoma subtypes are limited. In one study, VEGF expression differed in the subtypes, thus implicating different mechanisms of VEGF expression and/or action (72). A therapeutic benefit of VEGF targeting in pituitary adenomas was recently demonstrated in an animal study (73). Anti-VEGF treatment resulted in inhibition of pituitary adenoma growth associated with decreased serum PRL levels in a mouse model of multiple endocrine neoplasia type-1. Additionally, inhibition of VEGF secretion was found to be associated with the therapeutic effect of somatostatin analogs on non-functioning pituitary adenomas (73). Because of contradictory findings, the utility of serum VEGF as a marker of pituitary tumor behavior remains unclear. The potential for VEGF as a marker of aggressive tumor behavior in pituitary neoplasms is inconclusive when compared with other types of neoplasms because the former often behave as benign, non-malignant entities.

MVD. Some biomarkers, such as CD31, CD34 and VEGF, are used for the assessment of tumoral MVD (67). It was shown by some studies that MVD of the anterior pituitary is lower than that of normal pituitary, whereas in others, some endocrine tumors had a higher MVD (67). Therefore, the correlation between the expression of VEGF and bFGF in pituitary tumor aggressiveness is uncertain. But some studies showed a positive relation between aggressive behavior of pituitary tumor and MVD (74-76). Findings of likely relations between pituitary tumor invasiveness and MVD are generally scarce (75). In addition, extensive variability exists in the findings of various studies which concentrated in tumor types and specific MVD values.

HIF-1α. Hypoxia-inducible factors (HIFs) are transcription factors that respond to changes in available oxygen in the cellular environment, specifically, to decreases in oxygen, or hypoxia (77). Most, if not all, oxygen-breathing species express the highly-conserved transcriptional complex HIF-1, which is a heterodimer composed of an alpha- and a beta-subunit, the latter being a constitutively expressed aryl hydrocarbon receptor nuclear translocator (ARNT) (78, 79). HIF-1 belongs to the aryl hydrocarbon receptor nuclear translocator (PER-ARNT-SIM PAS) subfamily of the basic helix-loop-helix family of transcription factors. The HIF signaling cascade mediates the effects of hypoxia, a state of low oxygen concentration on cells. Hypoxia often keeps cells from differentiating. However, it also promotes the formation of blood vessels, and is important for the formation of a vascular system in embryos, and tumors. Hypoxia in wounds also promotes the migration of keratinocytes and the restoration of the epithelium (80). HIFs play a central role in the regulation of metabolism of Man (81) and are vital to development. In mammals, deletion of the HIF-1 genes results in perinatal death. HIF-1 has been shown to be vital to chondrocyte survival, allowing these cells to adapt to low-oxygen conditions within the growth plates of bones.

The alpha subunits of HIF are hydroxylated at conserved proline residues by HIF prolyl-hydroxylases, allowing their recognition and ubiquination by the von Hippel-Lindau (VHL) E3 ubiquitin ligase, which labels them for rapid degradation by the proteasome (82). This occurs only under normoxic conditions. Under hypoxic conditions, HIF prolyl-hydroxylase is inhibited, since it utilizes oxygen as a co-substrate (83). Hypoxia also results in a buildup of succinate, due to inhibition of the electron transport chain in the mitochondria. The buildup of succinate further inhibits HIF prolyl-hydroxylase action, since it is an end-product of HIF hydroxylation. Similarly, inhibition of electron transfer in the succinate dehydrogenase complex due to mutations in the succinate dehydrogenase (SDH) gene B (SDHB) or succinate dehydrogenase (SDH) gene D (SDHD) can cause a build-up of succinate that inhibits HIF prolyl-hydroxylase, termed as pseudohypoxia under stabilizing HIF-1α. HIF-1α, when stabilized by hypoxic conditions, up-regulates several genes to promote cell survival under low-oxygen conditions. These include genes for glycolytic enzymes, which allow ATP

synthesis in an oxygen-independent manner, and VEGF, which promotes angiogenesis. HIF-1α acts by binding to HIF-responsive elements (HREs) in promoters that contain the sequence NCGTG. It has been shown that a *muscle KINASE-ANCHORING PROTEIN* (mAKAP) organized E3 ubiquitin ligases, affecting stability and positioning of HIF-1α inside its site of action in the nucleus. Depletion of mAKAP or disruption of its targeting to the perinuclear region altered the stability of HIF-1α and transcriptional activation of genes associated with hypoxia particularly in cardiomyocytes. This 'compartmentalization' of oxygen-sensitive signaling components may change the reaction to hypoxia (84).

The advanced knowledge of the molecular regulatory mechanisms of HIF-1α activity under hypoxic conditions contrasts sharply with the paucity of information on the mechanistic and functional aspects governing nuclear factor κB (NF-κB)-mediated HIF-1α regulation under normoxic conditions. However, HIF-1α stabilization is also found in non-hypoxic conditions through a mechanism which was recently revealed. It was shown that NF-κB is a direct modulator of HIF-1α expression in the presence of normal oxygen pressure. Small interfering RNA (siRNA) studies for individual NF-κB members revealed differential effects on *HIF-1α* mRNA levels, indicating that NF-κB can regulate basal HIF-1α expression. It was also shown that when endogenous NF-κB is induced by tumor necrosis factor α (TNFα) administration, HIF-1α levels change in an NF-κB-dependent manner (85).

Recently, several drugs have been developed which act as selective HIF prolyl-hydroxylase inhibitors (86). The most notable of these include FibroGen's compounds FG-2216 and FG-4592, both of which are intended as orally acting drugs for the treatment of forms of anemia. By inhibiting HIF prolyl-hydroxylase, the activity of HIF-1α in the bloodstream is prolonged, which results in an increase in endogenous production of erythropoietin. Both of these drugs made it through to phase II clinical trials, but these were suspended temporarily in May 2007 following the death of a trial participant from fulminant hepatitis. However, it is unclear whether this death was caused by FG-2216. The hold on the study was lifted in early 2008 as the Food and Drug Administration (FDA) reviewed and approved a thorough response from FibroGen (87).

HIF activity is also involved in angiogenesis required for cancer tumor growth, so HIF inhibitors are also under investigation for anticancer effects (88). HIF-1α is up-regulated under hypoxic conditions and in turn up-regulates VEGF expression. This pathway is thought to be involved in the vascularization of tumors growing under hypoxic conditions (89). Kim *et al*. found no significant correlation between the expression of VEGF and HIF-1α; their co-localization was seen in only a few cells (89). Thus, hypoxia-induced VEGF expression may not be an important vasculogenic pathway in pituitary adenomas. Similarly, Vidal *et al*. showed that HIF-1α expression did not correlate with MVD (49), thus suggesting that despite HIF-1α–mediated regulation of VEGF in other tumor types, its expression in pituitary tumors may be affected by alternate pathways.

Examination of a series of pituitary tumors (n=155) for HIF-1α expression showed it to be limited to the nuclei of tumor and endothelial cells, with non-tumoral cells being immunonegative (49). No significant correlation was found between its expression and patient age, sex, or tumor size. With respect to tumor subtype, studies of HIF-1α expression have demonstrated significantly higher levels in GH- and PRL-secreting adenomas and carcinomas, whereas the lowest levels were detected in ACTH-secreting adenomas (89-91). The findings of elevated HIF-1α expression in pituitary carcinomas and its decreased expression in ACTH-secreting adenomas are of particular interest and highlight the need for further studies into its value as a predictive biomarker.

Matrix Metalloproteinases (MMPs)

Metalloproteinases (or metalloproteases) constitute a family of enzymes from the group of proteases, classified by the nature of the most prominent functional group in their active site. These are proteolytic enzymes whose catalytic mechanism involves a metal (92). Most metalloproteases are zinc-dependent, but some use cobalt. The metal ion is coordinated to the protein *via* three ligands. The ligands coordinating the metal ion can vary with histidine, glutamate, aspartate, lysine, and arginine. The fourth coordination position is taken up by a labile water molecule. There are two subgroups of metalloproteinases: exopeptidases, (metalloexopeptidases) and endopeptidases (metalloendopeptidases). Well-known metallo-endopeptidases include A Disintegrin And Metalloprotease (ADAM) proteins and matrix metalloproteinases. Treatment with chelating agents such as EDTA leads to their complete inactivation. EDTA is a metal chelator which removes zinc, which is essential for the enzyme's activity. They are also inhibited by the chelator orthophenanthroline (92).

MMPs are proteolytic enzymes that break down basement membranes and connective tissues, thus facilitating invasive growth (92). They do so by breaking down the extracellular matrix and selectively remodeling it (92). In a recent microarray analysis and gene clustering study, Hussaini *et al*. found a robust, eight-fold increase in MMP-9 expression in invasive, compared with non-invasive adenomas (93), a result in keeping with the findings of earlier studies (3, 92, 94, 95). Several studies established that increased expression and/or activity of MMP-9 and/or MMP-2 corresponded to an invasive tumor phenotype and higher radiological tumor grade (3, 66, 92, 94, 96). Yet other studies investigating a possible correlation between pituitary tumor invasiveness and MMP-9 expression failed to show any association (97-99). Other members of the MMP family, such as MMP-1, -2, and

-3, have also been shown to be differentially expressed in pituitary adenomas (3, 92, 97). The role of MMPs as clinicopathological markers of pituitary adenomas has not been established, despite considerable support for this notion. Discrepancies may be rooted in variability in definition of tumor parameters, especially of invasiveness, because this is often variably defined based on radiological, surgical, and/or microscopic findings. Nonetheless, MMPs offer much promise as predictors of tumor behavior. Standardization of approaches to the measurement of MMP levels and activity may clarify some of these contradictory findings.

Microarrays

MicroRNAs (miRNAs) are small 22-nucleotide-long, single-stranded, non-coding RNA molecules. They post-transcriptionally regulate the expression of downstream mRNAs by targeting the 3' untranslated regions (100, 101). After the discovery of miRNAs that form a class of conserved genes, hundreds of miRNA genes have been identified. So far more than 6,000 miRNAs encoded by virus, plant and animal species have been recorded in the miRBase bank (102, 103). A large class formed by miRNAs are negative gene regulators controlling a group of biological functions, *i.e.* cell proliferation, differentiation, signaling pathways, apoptosis and metabolism (104, 105). Additional evidence propose that some miRNAs might have oncogenic or tumor suppressor functions (106), and play an important role in tumorigenesis (107). Prior studies have shown that expression of miR-15a and miR-16-1 in pituitary adenomas is lower than that in the normal pituitary tissues. These markers could be potentially useful diagnostic markers, improving the classification of pituitary adenomas. However, the role of transcriptional regulation of miRNAs and their target genes in the pathogenesis of pituitary adenomas remains largely unknown (108).

A recent study has indicated that altered miRNA expression may be involved in GH-secreting pituitary adenoma transformation (108). Furthermore, some differentially expressed miRNAs are associated with tumor diameter, lanreotide treatment, and responsiveness to somatostatin analogs (SSA). Conclusively, these results will facilitate our understanding on the mechanism of SSA treatment for acromegaly. Further studies are needed to predict the targets of up-regulated and down-regulated miRNAs and their co-factors in pituitary adenomas. Studying the targets of de-regulated miRNAs may elucidate-molecular mechanisms involved in pituitary adenoma pathogenesis.

Pituitary Stem Cells

The pituitary gland is the master endocrine regulator in the human body. It plays an important role in such vital physiological processes as growth, reproduction, metabolism, and immune response. The adenohypophysis, the secretory anterior lobe of the gland, contains five different types of hormone-secreting cells: lactotrophs (secreting PRL), somatotrophs (secreting GH), corticotrophs (secreting ACTH), gonadotrophs (secreting FSH and LH), and thyrotrophs (secreting TSH). The pituitary gland of newborns already presents a full set of terminally differentiated hormone-producing cells (109, 110). However, the postnatal gland undergoes extensive remodeling during one's lifetime. Soon after birth, the adenohypophysis enters a phase of growth that results in a dramatic increase in the size of the gland (111). The adult pituitary gland has the ability to adapt its cellular composition in response to changing physiological conditions, and this ability is thought to be mediated *via* the hypothalamus. For instance, the number of GH-secreting cells doubles during puberty, whereas the number of PRL-secreting cells expands and contracts several-fold during pregnancy, lactation, and weaning (111). The pituitary gland also appears to repopulate cells after tissue loss (112, 113). Proposed mechanisms include mitoses of differentiated cells, trans-differentiation between phenotypes, and the differentiation of pituitary stem cells. However, there is no conclusive *in vivo* evidence that any of these processes actually occur (114).

The existence of pituitary stem cells in the adult pituitary gland is supported by such findings as postnatal proliferation, differentiation based on environmental alterations, and development of hormone-producing cells after specific lesions in the pituitary. Stem cell characteristics, including renewal, proliferation abilities, and the presence of stem cells markers, have been demonstrated in adult pituitary cells from mammals. However, the proliferative ability observed is so far limited, and the potential for differentiation into hormone-secreting cells remains to be conclusively proven (111-113). Stem cell markers have been detected in animal models of pituitary tumorigenesis; however, a direct connection with tumor formation has not been demonstrated. Research into the capacity of 'pituitary stem cells' to differentiate *in vitro* and *in vivo* will clarify the mechanisms for regulation of these cells. As pituitary stem cells are better-understood, clinical applications, such as the treatment of pituitary adenomas and the implantation of pituitary stem cells for hormonal deficiencies, may be developed (115).

Current evidence supports the role of stem cells in the repair and plasticity of different organs in the human body such as the heart and the brain (116-118). The stem cells in these organ systems display the fundamental characteristics of a stem cell. These characteristics include self-renewal capacity, lack of specialization, and pluripotency, with the ability to differentiate into different cell phenotypes. In

culture, stem cells form colonies of undifferentiated, pluripotent cells that contain unique stem cell surface, cytoplasmic, and nuclear markers. In addition, these cells characteristically form sphere-like structures which in the example of neuronal stem cells (NSCs) are called neurospheres. Similar to these other organ systems, the plasticity of the pituitary gland and its alterations during adult life may be secondary to the activity of adult stem cells present in the gland (112, 119-124). Stem cell markers such as SOX2 (sex-determining region Y-box 2) (123), nestin (124), SCA1 (stem cell antigen-1) (125), and CD13322 have been identified in subpopulations of cells in the adult pituitary gland of animal models. In addition, 'pituispheres' have been generated *in vitro* in these models (120, 122). These findings support a potential role for pituitary stem cells in adult pituitary plasticity.

The adenohypophysis contains not only hormone-producing cells but also a substantial proportion of cells that do not express hormonal markers (110, 111, 114). These non–hormone-secreting cells are called chromophobes. These cells do not stain with the periodic acid–Schiff stain because of the absence of secretory hormone-containing granules. The chromophobes were the first group of cells to be considered for the role of pituitary stem cell by Yoshimura *et al*. in 1969 (114). In their study, chromophobes were purified from one-year-old mice and transplanted into the hypophysiotrophic area of the hypothalamus after surgical resection of the animal's own pituitary gland. The authors reported that pituitary-like structures formed *via* proliferation and differentiation of chromophobes into acidophils and basophils.

In a follow-up study by Otsuka *et al*. chromophobes differentiated *in vitro* into mature acidophils and basophils after the addition of hypothalamic hormones (126). However, the authors were unable to demonstrate one of the fundamental characteristics of a stem cell: the capacity of a single-cell to originate more than one lineage of cells. Furthermore, the authors did not report whether the regenerated pituitary tissue was endocrinologically active in the animal models after transplantation (114). The failure to demonstrate pluripotency may be partially explained by the heterogeneity of the cell group classified as chromophobes. This group includes agranular cells such as follicular cells (FS) cells, marginal zone cells, degranulated hormonal cells, and mesenchymal and immune cells (112). The heterogeneous ability of agranular cells to differentiate into acidophils and basophils (114, 126) supports the hypothesis that chromophobes might display stem cell characteristics (112). However, only a small subpopulation of chromophobes actually displays these characteristics (112-114). Therefore, the individual study of such cells reflects the current trend in pituitary stem cell research in an attempt to identify this subpopulation.

The occurrence of plurihormonal and null cell-type adenomas and the minimal mitotic activity present in the hyperplastic pituitary gland support the hypothesis that pituitary stem cells are a potential cellular source of pituitary adenomas (127). Alterations in the hormonal environment might be associated with changes in the normal pattern of growth/differentiation of these cells and therefore promote pituitary tumorigenesis.

Most of the pituitary pathophysiological data has been obtained in animal models. The study of human pituitary tissue still presents several limitations, including the anatomic inaccessibility of the pituitary gland, lack of functional human cell lines in culture, paucity of reliable animal models, and unique murine tumor growth characteristics (127). For instance, the higher mitotic activity and expansion of murine pituitary tissue must be considered before the results are analyzed and extrapolated as being representative of the human pituitary gland (128). However, animal studies have provided important information about pituitary physiology and the mechanisms of pituitary tumorigenesis. We discuss which adult pituitary cell lineages might have a pituitary stem cell role and the potential participation of these cells in pituitary tumorigenesis. In addition, we present possible future clinical applications such as the development of new treatment strategies for pituitary adenomas and hormone deficiencies.

Folliculostellate cells (FSC). Ultrastructural features of follicular structures reveal elongate or stellate cells known as FSCs, attached to each other *via* terminal bars at their apical surface and by desmosomes at their lateral cell membranes that form major parts of the adenohypophysial parenchyma (129, 130). FCSs occupy an approximately central position within every acinus in the adult human gland. Electron microscopically, these specific cells are furnished with a small nucleus and inconspicuous nucleolus, numerous free cytoplasmic polyribosomes but scant rough endoplasmic reticulum (RER), small Golgi apparatus. Additionally, there might be a few intermediate filaments, and/or glycogen particles. Immunohistochemically, they are immunoreactive for S-100 and for glial fibrillary acidic protein (GFAP) (131-133). Unfortunately, these immunoreactivity patterns do not co-exist and are only temporary, as related to phases of the FSC life cycle. Some immunoreactivity was found to be associated with EMA and galectin-3 immunoreactivities (50, 134).

The unexpected diversity of functions, from hormone production to immune roles, coupled with morphological variations within FSC, compelled some investigators to assume a dual derivation of FSC as pituitary and hemopoetic (dendritic) cells (135). In an extensive study, formation of follicles within the pituitary primordium was noted as early as six weeks of gestation (136). At this time, the stubby villi fill most of the small lumina. Later (8-10 weeks of

gestation), the morphology of the follicles is not much different from that seen in the adult gland. In the fetal gland, the follicles may contain FSCs possessing a few small secretory granules, fulfilling the criteria of endocrine differentiation, but not more differentiated forms.

Different developmental phases of antenatal and postnatal life exhibit different architectural structures showing gradual evolution of the pituitary acinus. This is a late event starting close to term, and the pituitary in the newborn may exhibit only focal emergence of the subsequent microcompartmentalization. The newborn adenohypophysis contains no cells immunoreactive for S-100 or GFAP. Several GFAP-positive cells appear in the cleft area, within the small embryonic remnants of the Rathke's pouch. The late appearance of S-100 and GFAP immunoreactivities result most likely from the previously described mechanism (129). It was found that follicles are not stationary structures: new FSCs are formed by glandular cells around single-cell necroses by forming terminal bars and desmosomes, thereby isolating the debris (129). Subsequently, they dismantle their endocrine machinery, taking up the non-endocrine phenotype of FSC. This process was interpreted to be ubiquitous as well as reversible (137). This particular reversibility most probably may be explained as both the FSC becoming a progenitor null-cell, followed by differentiation to participate in tissue repair or in hyperplastic processes, and tumorigenesis (137).

As for extensive use of immunohistochemistry, electron microscopy, and immunoelectron microscopy led to the concept of an inflexible one cell–one hormone, thus five-cell-type model (138). Therefore, the use of modern techniques open new horizons in understanding of new adenoma types and previously undisclosed cell types belonging to or resident in the human adenohypophysis (138). Making use of modern techniques resulted in recognition of new adenoma types and previously undisclosed cell types belonging to or resident in human adenohypophysis. All the morphological, *in vitro* biochemical, and genetic data, the neoplastic potential make the small FSC more than just an equal member of the pituitary cell population: it emerges as a pluripotent pituitary-specific adult stem cell (137).

Microenvironment

In microinvasion and proliferation of pituitary tumors, cross-talk exists between intracellular pathways and complex microenvironmental factors, processes that can be modulated at various levels. The signaling pathways of growth, angiogenic factors and hormones are intricate; therefore, alterations induced upon key molecules can lead to aberrant proliferation. The demonstrated overactivity of protein kinase-B (AKT) and mitogen-activated protein (MAP) kinase (MAPK) pathways qualifies them as valuable targets for inhibition, mediated by somatostatin analogs. An increasing body of evidence suggests clinically significant implications of PTTG1 in correlation with aggressive phenotypes or survival rate, thus PTTG1 is an interesting candidate biomarker for malignancy, tumor staging and subsequent therapeutic interventions. Future work should focus on the understanding over the molecular mechanisms that control pituitary tumor development, where intracellular signaling molecules will constitute not only diagnostic/prognostic markers, but also novel therapeutic targets (139).

Inflammation and Tumor

Among pathogenetic mechanisms and markers outlined above, same research suggests that HIF induction in normoxia is likely to have serious consequences in disease settings with a chronic inflammatory component (85). It has also been documented that chronic inflammation is self-perpetuating and that it distorts the microenvironment as a result of aberrantly active transcription factors. Ultimately, alterations in growth factor, chemokine, cytokine and reactive oxygen species (ROS) balance occur within the cellular *milieu* that in turn provide the axis of growth and survival needed for *de novo* development of cancer and metastasis. The results of a recently published study have numerous implications for a number of pathologies where NF-kB and HIF-1 are de-regulated, including rheumatoid arthritis and cancer (85). Therefore, it is thought that understanding the cross talk between these two key transcription factors, NF-κB and HIF, will greatly enhance the process of drug development (85).

HIF activity is involved in angiogenesis required for cancer tumor growth, HIF inhibitors are, thus, under investigation for their anticancer effects (83, 140). (*e.g.* phenethyl isothiocyanate (141).

Tumor Heterogeneity

There is much evidence to support the notion that pituitary tumors are clonal lesions caused by intrinsic pituitary cell defects, most of which are based on X chromosome inactivation (142, 143). In support of this concept, it is unusual to find hyperplasia of the anterior pituitary gland surrounding an adenoma (which might be expected if the adenoma arose as the result of an external hormonal stimulus), although occasional examples have been described (47). Multiple synchronous adenomas have been described in the pituitary gland, including tumors of different cell types, although they are extremely rare (144-146). A multiclonal origin has been proposed for these tumors, and there is increasing evidence to suggest that at least some sporadic tumors may also be multiclonal (143, 147-149). Genetic studies of pituitary adenomas have revealed that most of the mutations that have been identified in other

malignancies are usually absent, and the molecular events leading to adenoma formation are thus still poorly understood (147, 150-153). Although pituitary adenomas occur in multiple endocrine neoplasia type-1 (MEN1) (154), loss of heterozygosity at the *MEN1* gene locus is uncommon in sporadic adenoma (150, 152). Similarly, although pituitary GH-expressing adenomas occur as part of the Carney complex, one of the genes responsible for encoding an enzyme for cAMP-dependent protein kinase type I-alpha regulatory subunit is (*PRKAR1A*), which does not appear to be involved in the development sporadic pituitary tumours (155). Deletions in the region of 13q14 have been identified in pituitary adenomas, suggesting the presence of a tumor suppressor gene at this locus (151, 152, 156). It has also been suggested that amplification of the transforming protein p21 (*HRAS*) and of the regulator gene that codes for a transcription factor (*CMYC*), and inactivation of the tumor suppressor genes Rb1, Tp53, and nucleoside diphosphate kinase An encoding metastasis suppressor gene *NM23,* may represent mechanisms by which pituitary tumors progress, but there is as yet no evidence of their consistent involvement in pituitary adenoma invasion or in pituitary carcinomas (147, 157-160). Certain cell-specific genetic abnormalities have been identified, up to 40% of GH-secreting adenomas have Gs protein (GSP) mutations, resulting in activation of the Gs α subunit (147, 148, 161). Inactivation of *p16* has been identified in up to 80% of adenomas (particularly in large tumors in two recent studies, possibly as a result of *CDKN2A* methylation (162, 163). A novel oncogene, *PTTG*, is overexpressed in a wide range of pituitary adenomas and immunohistochemistry for PTTG protein is positive in most adenomas, but absent from normal pituitary cells (53). The precise role of PTTG in oncogenesis is uncertain; PTTG may interact with FGF to stimulate angiogenesis, or it may activate p53 to cause apoptosis (53, 59). A recent large study found an association between *cyclin-D1* genotype and tumor grade in sporadic pituitary adenomas, (164) but the clinical relevance of this finding remains uncertain until it is reproduced in other populations with these tumors. A novel pituitary tumor-derived, N-terminally truncated isoform of FGF receptor-4, ptd-FGFR4, was identified. (165). ptd-FGFR4 is not expressed in normal pituitary tissues, and has a distinctive cytoplasmic residence. It has a transforming effect, both *in vivo* and *in vitro*, and targeted expression of ptd-FGFR4 in transgenic mice results in pituitary tumors that morphologically resemble PRL-secreting adenomas in humans, in the absence of PRL cell hyperplasia (165).

Conclusion

We explored various biomarkers which may be involved in pituitary gland tumor induction and progression. Their study is very useful because they can provide information related to tumor cell proliferation and behavior, and which might lead to therapeutic applications. We emphasize the need for more consistent tumor markers and definitions. A wide variety of molecules affect tumor development in the pituitary. Hence the unavoidable requirement to develop a wide variety of biomarkers, rather than reliance on single ones, has emerged. In conclusion, newer techniques including DNA and microRNA microarrays and recent data about tumor microenvironment and tumor heterogeneity will indisputably provide new candidates, important to pituitary tumorigenesis.

Acknowledgements

The Authors are grateful to the Jarislowsky and Lloyd Carr-Harris Foundations for their support.

References

1 Decock J, Paridaens R and Ye S: Genetic polymorphisms of matrix metalloproteinases in lung, breast and colorectal cancer. Clin Genet *73(3)*: 197-211, 2008.

2 Bamberger CM, Fehn M, Bamberger AM, Lüdecke DK, Beil FU, Saeger W and Schulte HM: Reduced expression levels of the cell-cycle inhibitor p27KIP1 in human pituitary adenomas. Eur J Endocrinol *140(3)*: 250-255, 1999.

3 Beaulieu E, Kachra Z, Mousseau N, Delbecchi L, Hardy J and Béliveau R: Matrix metalloproteinases and their inhibitors in human pituitary tumors. Neurosurgery *45(6)*: 1432-1440, 1999.

4 Fukui S, Nawashiro H, Otani N, Ooigawa H, Yano A, Nomura N, Tokumaru AM, Miyazawa T, Ohnuki A, Tsuzuki N, Katoh H, Ishihara S and Shima K: Vascular endothelial growth factor expression in pituitary adenomas. Acta Neurochir Suppl *86*: 519-521, 2003.

5 Hentschel SJ, McCutcheon E, Moore W and Durity FA: p53 and MIB-1 immunohistochemistry as predictors of the clinical behavior of nonfunctioning pituitary adenomas. Can J Neurol Sci *30(3)*: 215-219, 2003.

6 Hiyama H, Kubo O, Kawamata T, Ishizaki R and Hori T: Expression of cyclin kinase inhibitor p21/WAF1 protein in pituitary adenomas: Correlations with endocrine activity, but not cell proliferation. Acta Neurochir (Wien) *144(5)*: 481-488,2002.

7 Bloomer CW, Kenyon L, Hammond E, Hyslop T, Andrews DW, Curran WJ and Dicker AP: Cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) expression in human pituitary macroadenomas. Am J Clin Oncol *26(4)*: 75-80, 2003.

8 Jin L, Qian X, Kulig E, Sanno N, Scheithauer BW, Kovacs K, Young WF Jr and Lloyd RV: Transforming growth factor-beta, transforming growth factor-beta receptor II, and $p27^{KIP1}$ expression in nontumorous and neoplastic human pituitaries. Am J Pathol Aug *151(2)*: 509-519, 1997.

9 Onguru O, Casey MB, Kajita S, Nakamura N and Lloyd RV: Cyclooxygenase-2 and thromboxane synthase in non-endocrine and endocrine tumors: a review. Endocr Pathol *16(4)*: 253-277, 2005.

10 Thodou E, Argyrakos T and Kontogeorgos G: Galectin-3 as a marker distinguishing functioning from silent corticotroph adenomas. Hormones (Athens) *6(3)*: 227-232, 2007.

11 Wierinckx A, Auger C, Devauchelle P, Reynaud A, Chevallier P, Jan M, Perrin G, Fèvre-Montange M, Rey C, Figarella-Branger D, Raverot G, Belin MF, Lachuer J and Trouillas J: A diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors: Endocr Relat Cancer *14*: 887-900, 2007.

12 Salehi F, Agur A, Scheithauer BW, Kovacs K, Lloyd RV and Cusimano M: Biomarkers of pituitary: A review. (Part II). Neurosurgery *67(6)*: 1790-1798, 2010.

13 Vidal S, Horvath E, Kovacs K, Scheithauer BW, Lloyd RV and Kontogeorgos G: Ultrastructural features of apoptosis in human pituitary adenomas. Ultrastruct Pathol *25(2)*: 85-92, 2001.

14 Ibrahim AE, Pickering RM, Gawne-Cain ML, King S, Lees PD and Ellison DW: Indices of apoptosis and proliferation as potential prognostic markers in nonfunctioning pituitary adenomas. Clin Neuropathol *23(1)*: 8-15, 2004.

15 Kulig E, Jin L, Qian X, Horvath E, Kovacs K, Stefaneanu L, Scheithauer BW and Lloyd RV: Apoptosis in nontumorous and neoplastic human pituitaries: expression of the BCL-2 family of proteins. Am J Pathol *54(3)*: 767-774, 1999.

16 Kontogeorgos G, Sambaziotis D, Piaditis G and Karameris A: Apoptosis in human pituitary adenomas: A morphologic and *in situ* end-labeling study. Mod Pathol *10(9)*: 921-926, 1997.

17 Nakabayashi H, Sunada I and Hara M: Immunohistochemical analyses of cell cycle related proteins, apoptosis, and proliferation in pituitary adenomas. J Histochem Cytochem *49(9)*: 1193-1194, 2001.

18 Losa M, Barzaghi RL, Mortini P, Franzin A, Mangili F, Terreni MR and Giovanelli M: Determination of the proliferation and apoptotic index in adrenocorticotropin-secreting pituitary tumors: Comparison between micro- and macroadenomas. Am J Pathol *156(1)*: 245-251, 2000.

19 Sambaziotis D, Kapranos N and Kontogeorgos G: Correlation of BCL-2 and BAX with apoptosis in human pituitary adenomas. Pituitary *6(3)*: 127-133, 2003.

20 Green VL, White MC, Hipkin LJ, Jeffreys RV, Foy PM and Atkin SL: Apoptosis and *p53* suppressor gene protein expression in human anterior pituitary adenomas. Eur J Endocrinol *36(4)*: 382-387, 1997.

21 Ozer E, Canda MS, Ulukus C, Guray M and Erbayraktar S: Expression of BCL-2, BAX and *p53* proteins in pituitary adenomas: An immunohistochemical study. Tumori *89(1)*: 54-59, 2003.

22 Suzui H, Takahashi JA, Fukumoto M, Hashimoto N, Itoh N, Hatanaka M and Kikuchi H: Immunohistochemical study for basic fibroblast growth factor and fibroblast growth factor receptor I in pituitary adenomas. Neurosci Lett *171(1-2)*: 192-196, 1994.

23 Scheithauer BW, Gaffey TA, Lloyd RV, Sebo TJ, Kovacs KT, Horvath E, Yapicier O, Young WF Jr., Meyer FB, Kuroki T, Riehle DL abd Laws ER Jr.: Pathobiology of pituitary adenomas and carcinomas. Neurosurgery *59(2)*: 341-353; discussion 341-353, 2006.

24 Suliman M, Royds J, Cullen D, Timperley W, Powell T, Battersby R and Jones TH: MDM2 and the p53 pathway in human pituitary adenomas. Clin Endocrinol (Oxf) *54(3)*: 317-325, 2001.

25 Kontogeorgos G, Kapranos N, Thodou E, Sambaziotis D and Tsagarakis S: Immunocytochemical accumulation of p53 in corticotroph adenomas: Relationship with heat-shock proteins and apoptosis. Pituitary *1(3-4)*: 207-212, 1999.

26 Kontogeorgos G: Predictive markers of pituitary adenoma behavior. Neuroendocrinology *83*: 179-188, 2006.

27 Thapar K, Scheithauer BW, Kovacs K, Pernicone PJ and Laws ER Jr.: p53 expression in pituitary adenomas and carcinomas: Correlation with invasiveness and tumor growth fractions. Neurosurgery *38(4)*: 765-770, 1996.

28 Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S and Melmed S: p21(Cip1) restrains pituitary tumor growth. Proc Natl Acad Sci USA *105(45)*: 17498-17503, 2008.

29 Melmed S: Acromegaly pathogenesis and treatment. J Clin Invest 119(11): 3189-3202, 2009.

30 Lloyd RV, Jin L, Qian X and Kulig E: Aberrant $p27^{KIP1}$ expression in endocrine and other tumors. Am J Pathol *150(2)*: 401-407, 1997.

31 Lloyd RV, Erickson LA, Jin L, Kulig E, Qian X, Cheville JC and Scheithauer BW: $p27^{KIP1}$: A multifunctional cyclin-dependent kinase inhibitor with prognostic significance in human cancers. Am J Pathol *154(2)*: 313-323, 1999.

32 Toyoshima H and Hunter T: p27, a novel inhibitor of G^1 cyclin-Cdk protein kinase activity is related to p21. Cell *15;78(1)*: 67-74, 1994.

33 Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM, Kaushansky K and Roberts JM: A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27 (Kip1)-deficient mice. Cell *85(5)*: 733-744, 1996.

34 Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA and Koff A: Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell *85(5)*: 721-732, 1996.

35 Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY and Nakayama K: Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell *85(5)*: 707-720, 1999.

36 Klopfleisch R, and Gruber AD: Differential expression of cell cycle regulators p21, p27 and p53 in metastasizing canine mammary adenocarcinomas *versus* normal mammary glands. Res Vet Sci *87(1)*: 91-96, 2009.

37 Klopfleisch R, Schütze M and Gruber AD: Loss of p27 expression in canine mammary tumors and their metastases. Res Vet Sci *88(2)*: 300-303, 2010.

38 Zhao D, Tomono Y and Nose T: Expression of $p27^{KIP1}$ and Ki-67 in pituitary adenomas: An investigation of marker of adenoma invasiveness. Acta Neurochir (Wien) *141(2)*: 187-192, 1999.

39 Ikeda H, Yoshimoto T and Shida N: Molecular analysis of *p21* and *p27* genes in human pituitary adenomas. Br J Cancer *76(9)*: 1119-1123, 1997.

40 Takeuchi S, Koeffler HP, Hinton DR, Miyoshi I, Melmed S and Shimon I: Mutation and expression analysis of the cyclin-dependent kinase inhibitor gene $p27^{KIP1}$ in pituitary tumors. J Endocrinol *157(2)*: 337-341, 1998.

41 Korbonits M, Chahal HS, Kaltsas G, Jordan S, Urmanova Y, Khalimova Z, Harris PE, Farrell WE, Claret FX and Grossman AB: Expression of phosphorylated p27(KIP1) protein and JUN activation domain-binding protein 1 in human pituitary tumors. J Clin Endocrinol Metab *87(6)*: 2635-2643, 2002.

42 Champoux JJ: DNA topoisomerases: structure, function, and mechanism. Annu Rev Biochem *70*: 369-413, 2001.

43 Rybenkov VV, Ullsberger C, Vologodskii AV and Cozzarelli NR: Simplification of DNA topology below equilibrium values by type II topoisomerases. Science *277*: 690-693, 1997.

44 Capranico G, Kohn KW and Cozzarelli NR: Local sequence requirements for DNA cleavage by mammalian topoisomerase II in the presence of doxorubicin. Nucleic Acid Res *18*: 6611-6619, 1990.

45 Järvinen TA, Tanner M, Rantanen V, Bärlund M, Borg A, Grénman S and Isola J: Amplification and detection of topoisomerase II alpha associate with ERB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol *156*: 839-847, 2000.

46 Münscher A, Schmid M, Saeger W, Schreiber S and Lüdecke DK: *GH-, PRL-, POMC-, beta-FSH,* mRNA in gonadotroph adenomas of the pituitary by *in situ* hybridization in comparison with immunostaining and clinical data. Endocr Pathol *12*: 171-180, 2001,

47 Vidal S, Kovacs K, Horvath E, Rotondo F, Kuroki T, Lloyd RV and Scheithauer BW: Topoisomerase II alpha expression in pituitary adenomas and carcinomas in relation to tumor behavior. Mod Pathol *15*: 1205-1212, 2002.

48 Onguru O, Scheithauer BW, Kovacs K, Vidal S, Jin L, Zhang S, Ruebel KH and Lloyd RV: Analysis of Cox-2 and thromboxane synthase expression in pituitary adenomas and carcinomas. Endocr Pathol *15(1)*: 17-27, 2004.

49 Vidal S, Kovacs K, Bell D, Horvath E, Scheithauer BW and Lloyd RV: Cyclooxygenase-2 expression in human pituitary tumors. Cancer *97(11)*: 2814-2821, 2003.

50 Riss D, Jin L, Qian X, Bayliss J, Scheithauer BW, Young WF Jr, Vidal S, Kovacs K, Raz A and Lloyd RV: Differential expression of galectin-3 in pituitary tumors. Cancer Res *63(9)*: 2251-2255, 2003.

51 Pei L and Melmed S: Isolation and characterization of a pituitary tumor-transforming gene (PTTG). Mol Endocrinol *11(4)*: 433-441, 1997.

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

53 Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD and Melmed S: Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. J Clin Endocrinol Metab *84*: 761-767, 1999.

54 Zou H, McGarry TJ, Bernal T and Kirschner MW: Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. Science *285(5426)*: 418-422, 1999.

55 McCabe CJ, Boelaert K, Tannahill LA, Heaney AP, Stratford AL, Khaira JS, Hussain S, Sheppard MC, Franklyn JA and Gittoes NJ: Vascular endothelial growth factor, its receptor KDR/FLK-1, and pituitary tumor transforming gene in pituitary tumors. J Clin Endocrinol Metab *87(9)*: 4238-4244, 2002.

56 McCabe CJ, Khaira JS, Boelaert K, Heaney AP, Tannahill LA, Hussain S, Mitchell R, Olliff J, Sheppard MC, Franklyn JA and Gittoes NJ: Expression of pituitary tumour transforming gene (PTTG) and fibroblast growth factor-2 (FGF-2) in human pituitary adenomas: Relationships to clinical tumour behaviour. Clin Endocrinol (Oxf) *58(2)*: 141-150, 2003.

57 Chen L, Liu YS, Wang LS, Yin HG, Hou QT, Liu ZX, Chen LH and Ling F: Expression of pituitary tumor transforming gene, endostatin, and basic fibroblast growth factor mRNAs in invasive pituitary adenomas. Zhong Nan Da Xue Xue Bao Yi Xue Ba *29(6)*: 651-653, 666, 2004.

58 Filippella M, Galland F, Kujas M, Young J, Faggiano A, Lombardi G, Colao A, Meduri G and Chanson P: Pituitary tumour transforming gene (PTTG) expression correlates with the proliferative activity and recurrence status of pituitary adenomas: a clinical and immunohistochemical study. Clin Endocrinol (Oxf) *65(4)*: 536-543, 2006.

59 Hunter JA, Skelly RH, Aylwin SJ, Geddes JF, Evanson J, Besser GM, Monson JP and Burrin JM: The relationship between pituitary tumour transforming gene (PTTG) expression and *in vitro* hormone and vascular endothelial growth factor (VEGF) secretion from human pituitary adenomas. Eur J Endocrinol *148(2)*: 203-211, 2003.

60 Sáez C, Japón MA, Ramos-Morales F, Romero F, Segura DI, Tortolero M and Pintor-Toro JA: HPTTG is overexpressed in pituitary adenomas and other primary epithelial neoplasias. Oncogene *18(39)*: 5473-5476, 1999.

61 Minematsu T, Suzuki M, Sanno N, Takekoshi S, Teramoto A and Osamura RY: PTTG overexpression is correlated with angiogenesis in human pituitary adenomas. Endocr Pathol. *17(2)*: 143-153, 2006.

62 Ruebel KH, Leontovich AA, Jin L, Stilling GA, Zhang H, Qian X, Nakamura N, Scheithauer BW, Kovacs K and Lloyd RV: Patterns of gene expression in pituitary carcinomas and adenomas analyzed by high-density oligonucleotide arrays, reverse transcriptase-quantitative PCR, and protein expression: Endocrine *29*: 435-444, 2006.

63 Salehi F, Agur A, Scheithauer BW, Kovacs K, Lloyd RV and Cusimano M: Ki-67 in pituitary neoplasms: A review Part I. Neurosurgery *65;3*: 429-437, 2009.

64 Gozu Iliksu H, Bilgic B, Hazneci J, Sargin H, Erkal F, Sargin M, Sonmez B, Orbay E, Seker M, Bozbuga M and Bayindir C: Is Ki-67 index a useful labeling marker for invasion of pituitary adenomas? Turk J Endocrin Metab *4*: 107-113, 2005.

65 Bottoni A, Zatelli MC, Ferracin M, Tagliati F, Piccin D, Vignali C, Calin GA, Negrini M, Croce CM and Degli Uberti EC: Identification of differentially expressed microRNAs by microarray: A possible role for microRNA genes in pituitary adenomas. J Cell Physiol *210*: 370-377, 2007.

66 Gong J, Zhao Y, Abdel-Fattah R, Amos S, Xiao A, Lopes MB, Hussaini IM and Laws ER: Matrix metalloproteinase-9, a potential biological marker in invasive pituitary adenomas: Pituitary *11*: 37-48, 2008.

67 Yamada S and Takada K: Angiogenesis in pituitary adenomas. Microsc Res Tech *60(2)*: 236-243, 2003.

68 Niveiro M, Aranda FI, Peiró G, Alenda C and Picó A: Immunohistochemical analysis of tumor angiogenic factors in human pituitary adenomas. Hum Pathol *36(10)*: 1090-1095, 2005.

69 Pan LX, Chen ZP, Liu YS and Zhao JH: Magnetic resonance imaging and biological markers in pituitary adenomas with invasion of the cavernous sinus space. Hiroshima J Med Sci *53(2)*: 23-27, 2004.

70 Arita K, Kurisu K, Tominaga A, Sugiyama K, Eguchi K, Hama S, Yoshioka H, Yamasaki F and Kanou Y: Relationship between intratumoral hemorrhage and overexpression of vascular endothelial growth factor (VEGF). Hiroshima J Med Sci *53(2)*: 23-27, 2004.

71 Luchi T, Saeki N, Osato K and Yamaura A: Proliferation, vascular endothelial growth factor expression and cavernous sinus invasion in growth hormone secreting pituitary adenomas. Acta Neurochir (Wien) *142(12)*: 1345-1351, 2000.

72 Lloyd RV, Scheithauer BW, Kuroki T, Vidal S, Kovacs K and Stefaneanu L: Vascular endothelial growth factor (VEGF) expression in human pituitary adenomas and carcinomas. Endocr Pathol *10(3)*: 229-235, 1999.

73 Korsisaari N, Ross J, Wu X, Kowanetz M, Pal N, Hall L, Eastham-Anderson J, Forrest WF, Van Bruggen N, Peale FV and Ferrara N: Blocking vascular endothelial growth factor-A inhibits the growth of pituitary adenomas and lowers serum prolactin level in a mouse model of multiple endocrine neoplasia type 1. Clin Cancer Res *14(1)*: 249-258, 2008.

74 Jugenburg M, Kovacs K, Stefaneanu L and Scheithauer BW: Vasculature in non-tumorous hypophysis, pituitary adenomas and carcinomas: a quantitative morphologic study. Endocrinol Pathol *2*: 115-124, 1995.

75 Turner HE, Nagy Z, Gatter KC, Esiri MM, Harris AL and Wass JA: Angiogenesis in pituitary adenomas – relationship to endocrine function, treatment and outcome. J Endocrinology *165;(2)*: 475-481, 2000.

76 Vidal S, Kovacs K, Horvath E, Scheithauer BW, Kuroki T and Lloyd RV: Microvessel density in pituitary adenomas and carcinomas. Virch Arch *438;(6)*: 595-602, 2001.

77 Smith TG, Robbins PA and Ratcliffe PJ: The human side of hypoxia-inducible factor. Br J Haematol *141(3)*: 325-334, 2008.

78 Wang GL, Jiang BH, Rue EA and Semenza GL: Hypoxia-inducible factor 1 is a basic helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA *92(12)*: 5510-5514, 1995.

79 Jiang BH, Rue E, Wang GL, Roe R and Semenza GL: Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem *271(30)*: 17771-17778, 1996.

80 Benizri E, Ginouvès A and Berra E: The magic of the hypoxia-signaling cascade. Cell Mol Life Sci *65(7-8)*: 1133-1149, 2008.

81 Formenti F, Constantin-Teodosiu D, Emmanuel Y, Cheeseman J, Dorrington KL, Edwards LM, Humphreys SM, Lappin TR, McMullin MF, McNamara CJ, Mills W, Murphy JA, O'Connor DF, Percy MJ, Ratcliffe PJ, Smith TG, Treacy M, Frayn KN, Greenhaff PL,Karpe F, Clarke K and Robbins PA: Regulation of human metabolism by hypoxia-inducible factor. Proc Natl Acad Sci USA *107(28)*: 12722-12727, 2010.

82 Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER and Ratcliffe PJ: The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature *399(6733)*: 271-275, 1999.

83 Semenza GL: Hydroxylation of HIF-1: Oxygen sensing at the molecular level. Physiology (Bethesda) *19(4)*: 176-182, 2004.

84 Wong W, Goehring AS, Kapiloff MS, Langeberg LK and Scott JD: mAKAP compartmentalizes oxygen-dependent control of HIF-1alpha. Sci Signal *1(51)*: ra18, 2008.

85 van Uden P, Kenneth NS and Rocha S: Regulation of hypoxia-inducible factor-1alpha by NF-kappaB. Biochem J *412(3)*: 477-484, 2008.

86 Bruegge K, Jelkmann W and Metzen E: Hydroxylation of hypoxia-inducible transcription factors and chemical compounds targeting the HIF-alpha hydroxylases. Curr Med Chem *14(17)*: 1853-1862, 2007.

87 Hsieh MM, Linde NS, Wynter A, Metzger M, Wong C, Langsetmo I, Lin A, Smith R, Rodgers GP, Donahue RE, Klaus SJ and Tisdale JF: HIF prolyl hydroxylase inhibition results in endogenous erythropoietin induction, erythrocytosis, and modest fetal hemoglobin expression in rhesus macaques. Blood *110(6)*: 2140-2147, 2007.

88 Semenza GL: Evaluation of HIF-1 inhibitors as anticancer agents. Drug Discov Today *12(19-20)*: 853-859, 2007.

89 Kim K, Yoshida D and Teramoto A: Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in pituitary adenomas. Endocr Pathol *16(2)*: 115-121, 2005.

90 Vidal S, Horvath E, Kovacs K, Kuroki T, Lloyd RV and Scheithauer BW: Expression of hypoxia-inducible factor-1alpha (HIF-1alpha) in pituitary tumours. Histol Histopathol *18(3)*: 679-686, 2003.

91 Yoshida D, Kim K, Yamazaki M and Teramoto A: Expression of hypoxia-inducible factor 1alpha and cathepsin D in pituitary adenomas. Endocr Pathol *16(2)*: 123-131, 2005.

92 Liu W, Kunishio K, Matsumoto Y, Okada M and Nagao S: Matrix metalloproteinase-2 expression correlates with cavernous sinus invasion in pituitary adenomas. J Clin Neurosci *12(7)*: 791-794, 2005.

93 Hussaini IM, Trotter C, Zhao Y, Abdel-Fattah R, Amos S, Xiao A, Agi CU, Redpath GT, Fang Z, Leung GK, Lopes MB and Laws ER Jr.: Matrix metalloproteinase-9 is differentially expressed in nonfunctioning invasive and noninvasive pituitary adenomas and increases invasion in human pituitary adenoma cell line. Am J Pathol *170(1)*: 356-365, 2007.

94 Kawamoto H, Kawamoto K, Mizoue T, Uozumi T, Arita K and Kurisu K: Matrix metalloproteinase-9 secretion by human pituitary adenomas detected by cell immunoblot analysis. Acta Neurochir (Wien) *138(12)*: 1442-1448, 1996.

95 Kawamoto H, Uozumi T, Kawamoto K, Arita K, Yano T and Hirohata T: Type IV collagenase activity and cavernous sinus invasion in human pituitary adenomas. Acta Neurochir (Wien) *138(4)*: 390-395, 1996.

96 Turner HE, Nagy Z, Esiri MM, Harris AL and Wass JA: Role of matrix metalloproteinase 9 in pituitary tumor behavior. J Clin Endocrinol Metab *85(8)*: 2931-2935, 2000.

97 Knappe UJ, Hagel C, Lisboa BW, Wilczak W, Lüdecke DK and Saeger W: Expression of serine proteases and metallo-proteinases in human pituitary adenomas and anterior pituitary lobe tissue. Acta Neuropathol *106(5)*: 471-478, 2003.

98 Yamada S, Ohyama K, Taguchi M, Takeshita A, Morita K, Takano K and Sano T: A study of the correlation between morphological findings and biological activities in clinically nonfunctioning adenomas. Neurosurgery *61(3)*: 580-584, 2007.

99 Yokoyama S, Hirano H, Moroki K, Goto M, Imamura S and Kuratsu JI: Are nonfunctioning pituitary adenomas extending into the cavernous sinus aggressive and/or invasive? Neurosurgery *49(4)*: 857-862; discussion 862-853, 2001.

100 Nilsen TW: Mechanisms of microRNA-mediated gene regulation in animal cells. Trends Genet *23*: 243-249, 2007.

101 Stefani G and Slack FJ: Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol *9*: 219-230, 2008.

102 Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR and Ruvkun G: The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature *403*: 901-906, 2000.

103 Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Müller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E and Ruvkun G: Conservation of the sequence and temporal expression of let-7 heterochromic regulatory RNA. Nature *408*: 86-89, 2000.

104 Esquela-Kerscher A and Slack FJ: Oncomirs–microRNAs with a role in cancer. Nat Rev Cancer *6*: 259-269, 2006.

105 Kloosterman WP and Plasterk RH: The diverse functions of microRNAs in animal development and disease. Dev Cell *11*: 441-450, 2006.

106 Gartel AL and Kandel ES: miRNAs: Little known mediators of oncogenesis. Semin Cancer Biol *18*: 103-110, 2008.

107 Calin GA and Croce CM: MicroRNA-cancer connection: the beginning of a new tale. Cancer Res *66*: 7390-7394, 2006.

108 Mao ZG, He DS, Zhou J, Yao B, Xiao WW, Chen CH, Zhu YH and Wang HJ: Differential expression of microRNAs in GH-secreting pituitary adenomas. Diagn Pathol *7(5)*: 79, 2010.

109 Rosenfeld MG, Briata P, Dasen J, Gleiberman AS, Kioussi C, Lin C, O'Connell SM, Ryan A, Szeto DP and Treier M: Multistep signaling and transcriptional requirements for pituitary organogenesis *in vivo*. Recent Prog Horm Res *55*: 1-13, 2000.

110 Scully KM and Rosenfeld MG: Pituitary development: Regulatory codes in mammalian organogenesis. Science. *295(5563)*: 2231-2235, 2002.

111 Levy A: Stem cells, hormones and pituitary adenomas. J Neuroendocrinol *20(1)*: 139-140, 2008.

112 Vankelecom H: Stem cells in the postnatal pituitary? Neuroendocrinology *85(2)*: 110-130, 2007.

113 Vankelecom H: Non-hormonal cell types in the pituitary candidating for stem cell. Semin Cell Dev Biol *18(4)*: 559-570, 2007.

114 Yoshimura F, Harumiya K, Ishikawa H and Otsuka Y: Differentiation of isolated chromophobes into acidophils or basophils when transplanted into the hypophysiotrophic area of hypothalamus. Endocrinol Jpn *16(5)*: 531-540, 1969.

115 Almeida JPC, Sherman JH, Salvatori R and Quiñones-Hinojosa A: Pituitary stem cells: Review of the literature and current understanding. Neurosurgery *67*: 770-780, 2010.

116 Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B and Anversa P: Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell *114(6)*: 763-776, 2003.

117 Rando TA: Stem cells, ageing and the quest for immortality. Nature *441(7097)*: 1080-1086, 2006.

118 Quinones-Hinojosa A and Chaichana K: The human subventricular zone: A source of new cells and a potential source of brain tumors. Exp Neurol *205(2)*: 313-324, 2007.

119 Levy A: Physiological implications of pituitary trophic activity. J Endocrinol *174(2)*: 147-155, 2002.

120 Borrelli E, Heyman RA, Arias C, Sawchenko PE and Evans RM. Transgenic mice with inducible dwarfism. Nature *339(6225)*: 538-541, 1989.

121 Nolan LA, Kavanagh E, Lightman SL and Levy A: Anterior pituitary cell population control: basal cell turnover and the effects of adrenalectomy and dexamethasone treatment. J Neuroendocrinol. 10(3):207-215, 1998.

122 Wilson DB: Distribution of ^{3}H-thymidine in the postnatal hypophysis of the C57BL mouse. Acta Anat (Basel) *126(2)*: 121-126, 1986.

123 Fauquier T, Rizzoti K, Dattani M, Lovell-Badge R and Robinson IC: SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland. Proc Natl Acad Sci USA *105(8)*: 2907-2912, 2008.

124 Gleiberman AS, Michurina T, Encinas JM, Roig JL, Krasnov P, Balordi F, Fishell G, Rosenfeld MG and Enikolopov G: Genetic approaches identify adult pituitary stem cells. Proc Natl Acad Sci USA *105(17):* 6332-6337, 2008.

125 Chen J, Hersmus N, Van Duppen V, Caesens P, Denef C and Vankelecom H: The adult pituitary contains a cell population displaying stem/progenitor cell and early embryonic characteristics. Endocrinology *146(9)*: 3985-3998, 2005.

126 Otsuka Y, Ishikawa H, Omoto T, Takasaki Y and Yoshimura F: Effect of CRF on the morphological and functional differentiation of the cultured chromophobes isolated from rat anterior pituitaries. Endocrinol Jpn *18(2)*: 133-153, 1971.

127 Melmed S: Mechanisms for pituitary tumorigenesis: The plastic pituitary. J Clin Invest *112(11)*: 1603-1618, 2003.

128 Mogi C, Miyai S, Nishimura Y, Fukuro H, Yokoyama K, Takaki A and Inoue K: Differentiation of skeletal muscle from pituitary folliculo-stellate cells and endocrine progenitor cells. Exp Cell Res. 292(2):288-294, 2004.

129 Horvath E, Kovacs K, Penz G and Ezrin C: Origin, possible function and fate of 'follicular cells' in the anterior lobe of the human pituitary. Am J Pathol *77(2)*: 199-212, 1974.

130 Horvath E and Kovacs K: Folliculo-stellate cells of the human pituitary: A type of adult stem cell? Ultrastruct Pathol *26*: 219-228, 2002.

131 Cocchia D and Miani N: Immunocytochemical localization of the brain-specific S-100 protein in the pituitary gland of adult rat. J Neurocytol *9*: 771-782, 1980.

132 Nakajima T, Yamaguchi H and Takahashi K: S-100 protein in folliculo-stellate cells of the rat anterior lobe. Brain Res *191*: 523-531, 1980.

133 Velasco ME, Roessman U and Gambetti P: The presence of glial acidic protein in the human pituitary gland. J Neuropathol Exp Neurol *41*: 150-163, 1982.

134 Roncaroli F, Scheithauer BW, Cenacchi G, Horvath E, Kovacs K, Lloyd RV, Abell-Aleff P, Santi M and Yates AJ: 'Spindle cell oncocytoma' of the adenohypophysis: A tumor of folliculostellate cells? Am J Surg Pathol *26*: 1048-1055, 2002.

135 Allaerts W, Fluitsma DM, Hoefsmit EC, Jeucken PH, Morreau H, Bosman FT and Drexhage HA: Immunohistochemical, morphological and ultrastructural resemblance between dendritic cells and folliculo-stellate cells in normal human and rat anterior pituitaries. J Neuroendocrinol *8*: 17-29, 1996.

136 Asa SL, Kovacs K, Horvath E, Losinski NE, Laszlo FA, Domokos I and Halliday WC: Human fetal adenohypophysis: electron microscopic and ultrastructural immunocytochemical analysis. Neuroendocrinology *48*: 423-431, 1988.

137 Horvath E, Coire IC, Kovacs K and Smyth HS: Folliculo-stellate cells of the human pituitary as adult stem cells: Examples of their neoplastic potential. Ultrastruct Pathol *34*: 133-139, 2010.

138 Horvath E, Scheithauer BW, Kovacs K and Lloyd RV: Hypothalamus and pituitary. In: Greendfield's Neuropathology, Vol 1, 7th ed. (Graham DI and Lantos PL, (eds.)) New York: Arnold, pp. 983-1051, 2002.

139 Tanase CP, Neagu M and Albulescu R: Key signaling molecules in pituitary tumors. Exp Rev of Mole Diagn *9(8)*: 859-877, 2009.
140 Melillo G: Inhibiting hypoxia-inducible factor 1 for cancer therapy. Mol Cancer Res *4(9)*: 601-605, 2006.
141 Syed Alwi SS, Cavell BE, Telang U, Morris ME, Parry BM and Packham G: *In vivo* modulation of 4E binding protein 1 (4E-BP1) phosphorylation by watercress: a pilot study. Br J Nutr *104(9)*: 1288-1296, 2010.
142 Chu I, Hengst L and Slingerland JM: The CDK inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev Cancer *8*: 253-267, 2008.
143 Clayton RN and Farrell WE: Clonality of pituitary tumours: More complicated than initially envisaged? Brain Pathol *11*: 313-327, 2001.
144 Vidal S, Horvath E, Syro LV, Uribe H, Cohen S and Kovacs K: Prolactin-producing pituitary adenoma associated with prolactin cell hyperplasia. Endocrinol Pathol *13*: 157-165, 2002.
145 Gale RE: Evaluation of clonality in myeloid stem-cell disorders. Semin Hematol *36(4)*: 361-372, 1999.
146 Santarosa M and Ashworth A: Haploinsufficiency for tumor suppressor genes: When you don't need to go all the way. Biochim Biophys Acta *1654(2)*: 105-122, 2004.
147 Asa SL and Ezzat S: The pathogenesis of pituitary tumours. Nat Rev Cancer *2*: 836-849, 2002.
148 Faglia G and Spada A: Genesis of pituitary adenomas: state of the art. J Neurooncol *54*: 95-110, 2001.
149 Buch H, El-Hadd T, Bicknell J, Simpson DJ, Farrell WE and Clayton RN: Pituitary tumours are multiclonal from the outset: Evidence from a case with dural metastases. Clin Endocrinol (Oxf) *56*: 817-822, 2002.
150 Boggild MD, Jenkinson S, Pistorello M, Boscaro M, Scanarini M, McTernan P, Perrett CW, Thakker RV and Clayton RN: Molecular genetic studies of sporadic pituitary tumors. J Clin Endocrinol Metab *78*: 387-393, 1994.
151 Farrell WE and Clayton RN. Molecular pathogenesis of pituitary tumors. Front Neuroendocrinol *21*: 174-198, 2000.
152 Lloyd RV: Molecular pathology of pituitary adenomas. J Neurooncol *54*: 111-119, 2001.
153 Nam DH, Song SY, Park K, Kim MH, Suh YL, Lee JI, Kim JS, Hong SC, Shin HJ, Park K, Eoh W and Kim JH: Clinical significance of molecular genetic changes in invasive pituitary adenomas. Exp Mol Med *33*: 111-116, 2001.
154 Komminoth P, Heitz PU and Kloppel G: Pathology of MEN-1: Morphology, clinicopathologic correlations and tumor development. J Int Med *243*: 455-464, 1998.
155 Sandrini F, Kirschner LS, Bei T, Farmakidis C, Yasufuku-Takano J, Takano K, Prezant TR, Marx SJ, Farrell WE, Clayton RN, Groussin L, Bertherat J and Stratakis CA: *PRKAR1A*, one of the Carney complex genes, and its locus (17q22–24) are rarely altered in pituitary tumours outside the Carney complex. J Med Genet *39(12)*: e78, 2002.
156 Alexander JM: Tumor suppressor loss in pituitary tumors. Brain Pathol *11*: 342-355, 2001.
157 Yu R and Melmed S: Oncogene activation in pituitary tumors. Brain Pathol *11*: 328-341, 2001.
158 Karga HJ, Alexander JM, Hedley-Whyte ET, Klibanski A and Jameson JL: Ras mutations in human pituitary tumors. J Clin Endocrinol Metab *74*: 914-919, 1992.
159 Suhardja A, Kovacs K and Rutka J: Genetic basis of pituitary adenoma invasiveness: A review. J Neurooncol *52*: 195-204, 2001.
160 Pei L, Melmed S, Scheithauer B, Kovacs K and Prager D: *H-Ras* mutations in human pituitary carcinoma metastases. J Clin Endocrinol Metab *78*: 842-846, 1994.
161 Spada A and Vallar L: G-Protein oncogenes in acromegaly. Horm Res *38*: 90-93, 1992.
162 Ruebel KH, Jin L, Zhang S, Scheithauer BW and Lloyd RV: Inactivation of the *p16* gene in human pituitary non-functioning tumors by hypermethylation is more common in null cell adenomas. Endocr Pathol *12*: 281-289, 2001.
163 Seemann N, Kuhn D, Wrocklage C, Keyvani K, Hackl W, Buchfelder M, Fahlbusch R and Paulus W: CDKN2A/p16 inactivation is related to pituitary adenoma type and size. J Pathol *193*: 491-497, 2001.
164 Simpson DJ, Fryer AA, Grossman AB, Wass JA, Pfeifer M, Kros JM, Clayton RN and Farrell WE: Cyclin D1 (CCND1) genotype is associated with tumor grade in sporadic pituitary adenomas. Carcinogenesis *22*: 1801-1807, 2001.
165 Ezzat S, Zheng L, Zhu XF, Wu GE and Asa SL: Targeted expression of a human pituitary-derived isoform of FGF receptor-4 recapitulates pituitary tumorigenesis. J Clin Invest *109*: 69-78, 2002.

Received July 17, 2012
Revised September 16, 2012
Accepted September 20, 2012