Biomarkers of Pituitary Neoplasms

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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,
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 Adrenocorticotropic 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, Kontogeorgos 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 pro-apoptotic 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 pro-apoptotic 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, ‘thannatosomes’ 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 inexplicable. 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 have 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 p27kip1 gene has a DNA sequence similar to other members of the ‘CIP/KIP’ family which include the p21cip1/waf1 and p57kip2 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, p27KIP1 binds to cyclin-D either alone, or when complexed to its catalytic subunit CDK4. In doing so, p27KIP1 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 p27KIP1 inhibit 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 p27KIP1 protein typically cause cells to arrest in the G1 phase of the cell cycle. Likewise, p27KIP1 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 p27KIP1 levels inside a cell. For example, levels of p27KIP1 increase when transforming growth factor (TGF) is present outside of epithelial cells, causing growth arrest (32). In contrast, interleukin (IL-2) causes p27KIP1 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 immunoreactivity 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, which would require the two strands to be unwound 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 G2 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.
investigated its potential links to other, newer biomarkers. marker in the study of pituitary tumors; many studies
Proliferative Marker Ki-67
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. 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-1a. 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. Hifoxia 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-1a. HIF-1a, 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 NGCGT. 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 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 metalloendopeptidases 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
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 CD133 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 adenohypophysis 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 undiscovered 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 undiscovered 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, some 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-κB 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 MENI 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 (PRKARIA), 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.

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