Biomarkers of Pituitary Neoplasms: a Review (Part II)

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Abstract

Several new markers have come to attention based upon their capacity to predict the clinicopathological behavior of pituitary neoplasms; these markers have shown potential to correlate with tumour subtype and size, as well as patient age and gender. These various markers are involved in a host of cellular functions, including cell cycle progression, cell proliferation, apoptosis, cell adhesion and tumour vascularity. In this second companion article to our first review of Ki-67 as a marker of pituitary adenomas, we present and analyze the literature regarding matrix metalloproteinase (MMPs) and their inhibitors (TIMPs), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and its receptor (FGFR), apoptotic markers and p53, as well as cyclooxygenase 2 (Cox-2), Galectin-3, and pituitary tumour transforming gene (PTTG). Some of these markers, such as FGF and FGFR as well as MMPs show particular promise in their ability to identify pituitary tumours that behave in an aggressive manner. We suggest the need for uniform design and application of methods as well as standardized criteria for the interpretation of results. Only such uniform approach will establish clinicopathologic utility of emerging markers.

Running Titles: Biomarkers in pituitary tumours

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Introduction

The management of pituitary tumours is complicated by difficulty predicting their clinical course. Several markers are thought to be of predictive value with regards to clinicopathologic parameters. The most widely utilized marker of tumour behaviour is Ki-67, an indicator of cell proliferation and the subject of our companion publication. However, the utility of Ki-67 as a marker of pituitary neoplasm behaviour is debatable, as several studies have shown contradictory findings with respect to patient age and gender, tumour size and subtype as well as the ability of Ki-67 staining to predict invasiveness and likelihood of recurrence. Thus, the search for new markers to predict the behavior of pituitary tumours continues. Some markers have shown promise. Established examples include matrix metalloproteinase’s (MMPs), p53 and the apoptotic index, the inhibitory cell cycle proteins, p27 and p21, and such markers of microvascular density as vascular endothelial growth factor (VEGF) (2,3,13,16,17). Two newer markers include cyclooxygenase II (Cox-2) and Galectin-3 (4,23,48,65,74). As in the case of Ki-67 staining and its correlation with clinicopathologic parameters, early results of the studies using these other markers have also been inconsistent. For example, whereas in other tumour types such as lung and colorectal cancer, fairly uniform consensus has been reached regarding the significance of expression of MMPs (9), this is not true of pituitary tumours, the majority of which are benign neoplasms. Similarly, whereas increased microvascular density is associated with aggressive behaviour in many non-endocrine tumours (6), the same does not apply to pituitary and other endocrine neoplasms.

These published inconsistencies in results may in part be due to variations in definition of invasion. In some studies its presence or absence is solely based on pre-operative radiological
and/or intra-operative surgical findings, whereas others define it as histological dural invasion. Another factor may be the inclusion of a variety of pituitary subtypes, instead of single or of distinct pathologic tumour types. This “mixed bag” approach to the study of often too small a series precludes meaningful conclusions. For example, some markers are restricted to specific cell types, such as Galectin-3 which is expressed by PRL and ACTH, but not by most other types (23). It may be that various hormonal and functional subtypes (functioning vs. non-hormone secreting tumours) exhibit differing marker and molecular genetic profiles. Thus, although some of the emerging biomarkers show promise to have predictive value in pituitary tumours, the need for uniform inclusion criteria and study design is discussed. Here, we review a number of markers of pituitary adenomas with respect to their strength as predictors of tumour behavior.

Markers of Tumour Angiogenesis

Angiogenesis is defined as the formation of new blood vessels from existing ones and is associated with tumour progression (75). It is axiomatic that tumour growth requires neovascularization in order to supply tumour cells with necessary nutrients and oxygen. Therefore, tumour vascularity is often associated with tumour growth, aggressive behaviour and metastatic potential (75). The utility of markers of angiogenesis as prognostic indicators of pituitary tumours are addressed by several studies investigating microvascular density (MVD), as well as expression of VEGF, EGF, FGF, Cox-2 and HIF-1 alpha.

Microvascular Density (MVD)
Tumoural microvascular density (MVD) is often assessed using such endothelial cell markers as CD31, CD34 and VEGF (75). Surprisingly, studies have shown that anterior pituitary tumours have lower MVD than normal pituitary tissue, in contrast to tumours of other non-endocrine organs (75). Thus, the relationship between the expression of such angiogenic factors as VEGF and basic fibroblast growth factor (bFGF) in pituitary tumour aggressiveness is unclear. It is of note that few studies have demonstrated a positive correlation between pituitary tumour aggressive behavior and MVD (25, 67, 72).

Similarly, conclusions regarding vascularity in pituitary carcinomas are inconsistent. Higher MVDs in pituitary carcinomas as compared to adenomas have been reported in several studies (25, 67, 72). Jugenburg et al. found higher MVD in carcinomas only when hotspots (focally densely vascular areas) were selected for quantification, no overall increase in vascularity of carcinomas being demonstrated as compared to adenomas (25). Although it is speculated that the various pituitary tumour subtypes may have differences in vascular marker expression, reported findings do not support this (69). Turner et al. described significant differences in MVD between invasive and non-invasive PRL adenomas but not so in GH and ACTH adenomas (68). They also found significantly lower MVDs in ACTH-producing adenomas and in microprolactinomas than other secreting and non-secreting tumour types (68).

Additionally, microprolactinomas demonstrated lower MVDs than macroprolactinomas, a finding perhaps related to higher Ki-67 LI in the latter (68). In contrast, Jugenberg et al. found MVDs to be highest in untreated PRL-adenomas and lowest in GH adenomas; these differences were not however statistically significant (25). Still different findings were reported by Niveiro et al. who found that TSH adenomas had the highest MVDs of all adenoma subtypes (47). Thus, MVD by itself is not a significant indicator of pituitary tumour behavior.
In sum, findings of possible correlations between MVD and pituitary tumour invasiveness are scarce (67). Similarly, much variability exists in the results of studies that focused upon MVD correlations with tumour subtypes.

**Vascular endothelial growth factor (VEGF)**

Vascular endothelial growth factor (VEGF) is an important angiogenic factor that mediates endothelial cell proliferation as well as permeability and motility. Its expression is related to tumour angiogenesis and often to aggressive behaviour (75). Findings of VEGF correlations with tumour invasiveness and proliferation (Table 1) are inconsistent, indicating that VEGF may not directly contribute to tumoural invasion, but may regulate pathways that do increase tumour volume or mediate invasiveness. This notion is supported by the observation that VEGF expression is not strictly associated with endothelium and vessels, but is expressed by adenoma cells as well (13).

Expression of VEGF receptors in pituitary adenomas has also been investigated. For example, higher expression of Flk-1 (fetal liver kinase-1), a form of VEGF receptor that mediates mitogenesis and affects endothelial cell morphology, was associated with extrasellar extension (42). Furthermore, Flk-1 was significantly higher in non-functioning as compared to functioning tumours (42). 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 (39).

A therapeutic benefit of VEGF targeting in pituitary adenomas was recently demonstrated in an animal study (34). Anti-VEGF treatment resulted in inhibition of pituitary
adenomas growth associated with decreased serum prolactin levels in a mouse model of multiple endocrine neoplasia type 1 (MEN1). Additionally, inhibition of VEGF secretion was found to be associated with the therapeutic effect of somatostatin analogs upon non-functioning pituitary adenomas (34). Nonetheless, due to contrast in findings, the utility of serum VEGF as a marker of pituitary tumour behavior remains unclear.

**FGF and EGF and their receptors**

Studies have shown associations between pituitary tumour behaviour and the expression of both bFGF (basic fibroblast growth factor), a well-characterized angiogenic growth factor, and its receptor, bFGFR (11, 54) (Table 2). Most investigations of EGFR expression in various pituitary tumour subtypes have shown it to be lower in non-functioning than in endocrinologically functioning adenomas (Table 3) (7, 31, 36, 50). EGFR expression was also shown to be significantly higher in ACTH- than in GH- or PRL-producing adenomas (64), a result suggesting that EGFR may be involved in the pathogenesis of ACTH adenomas.

Most studies have shown a good correlation between bFGF expression and clinicopathologic parameters including pituitary tumour maximal diameter and invasiveness, as well as patient outcome. In addition, expression of EGFR in pituitary tumours has often been shown to be a good predictor of tumour invasiveness.

**Hypoxia-inducible factor 1-alpha (HIF-1-alpha)**
HIF-1-alpha is upregulated under hypoxic conditions, and in turn upregulates VEGF. This pathway is thought to be involved in vascularization of tumours growing under hypoxic conditions (28). Interestingly, Kim et al, found no significant correlation between the expression of VEGF and HIF-1-alpha; their colocalization was seen in only a few cells (28). Thus, hypoxia-induced VEGF expression may not be an important vasculogenic pathway in pituitary adenomas (28). Similarly, Vidal et al. showed that HIF-1-alpha expression did not correlate with MVD, thus suggesting that despite HIF-1-alpha-mediated regulation of VEGF in other tumour types, its expression in pituitary tumours may be affected by alternate pathways (70).

Examination of a series of pituitary tumours (n=155) for HIF-1-alpha expression showed it to be limited to the nuclei of tumour and endothelial cells, non-tumoural cells being immunonegative (70). No significant correlation was found between its expression and patient age, gender, or tumour size. With respect to tumour subtype, studies of HIF-1 alpha expression have demonstrated significantly higher levels in GH (28,70), and PRL (79) adenomas as well as carcinomas (28, 70), while lowest levels were detected in ACTH adenomas (28, 70, 78). The findings of elevated HIF-1-alpha expression in pituitary carcinomas and its decreased expression in ACTH adenomas are of particular interest and highlight the need for further studies into its value as a predictive marker.

Cyclooxygenase-2 (Cox-2)

Cox-2, a key enzyme mediating prostoglandin synthesis, is involved in not only inflammatory responses, but is implicated in tumour invasiveness and angiogenesis (48, 49). Its expression in pituitary tumours has been recently demonstrated. Increased Cox-2 expression was particularly evident in pituitary carcinomas, as compared to adenomas and normal pituitary, thus suggesting a role significant in tumour progression. Onguru et al, found increased Cox-2
expression in functioning versus non-functioning tumours, both of which had lower levels of Cox-2 than did carcinomas (48, 49) (Table 4). Bloomer et al. found Cox-2 expression in 83% of 30 pituitary tumours (4). Its expression was significantly associated with that of LH and TSH (4). In contrast, the larger series of 164 pituitary tumours of Vidal et al. found that GH, PRL, TSH, and female gonadotrophs to express lower COX-2 levels than male gonadotrophs, oncocyctic and non-oncocytic null cell adenomas (71). The results of this study should focus analyses on several clinical variables including gender in gonadotrophic tumours, as gonadotrophs, compared to other pituitary neoplasms, express higher levels of Cox-2 (4, 71). There is also a significant association between COX-2 expression and patient age; no correlations were noted with patient sex, or with tumour size and invasiveness (71). Cox-2 expression did however show a strong correlation with MVD.

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases are proteolytic enzymes that break down basement membrane and connective tissue, thus facilitating invasive growth (37). They do so by breaking down extracellular matrix and selectively remodelling it (37). In a recent microarray analysis and gene clustering study, Hussaini et al found a robust, eightfold increase in MMP-9 expression in invasive as compared to non-invasive adenomas (19), a result in keeping with the findings of
earlier studies (3, 26, 27, 37, 66) (Table 5). Several studies have established that increased expression and/or activity of MMP-9 (14, 26, 27) and/or MMP-2 (37) correspond to invasive tumour phenotype, as well as higher radiological tumour grade (3). Yet other studies investigating a possible correlation between pituitary tumour invasiveness and MMP-9 expression failed to show an association (29, 76, 77) (Table 5).

Other members of the matrix metalloproteinase family, such as MMP1, 2 and 3 have also been shown to be expressed differentially in pituitary adenomas (3, 29, 37) (Table 5). Clearly, the role of MMP as a clinico-pathological marker of pituitary adenomas is not established yet, despite considerable support for this notion. Discrepancies may be rooted in variability in definition of tumour parameters, especially of invasiveness, as it is often variably defined based on radiologic, operative, and/or microscopic findings. Nonetheless, MMPs offer much promise as predictors of tumour behaviour. Standardization of approaches to the measurement of MMP levels and activity may clarify some of these contradictory findings.

**Markers of proliferation and cell survival**

**P27**

P27 kip1 (p27), a cyclin-dependent kinase inhibitor, is involved in regulation of cell cycle progression (2, 38, 40). In the earliest study of p27 in human pituitaries, Lloyd et al. found decreased p27 expression in pituitary neoplasms as compared to the normal gland (38, 40). As
expected, p27 immunoexpression was inversely correlated with the staining for the proliferation marker Ki-67, thus suggesting that p27 is an additional predictive marker of pituitary tumour behaviour (38, 40). Similarly, several studies found significantly lower p27 levels in non-functioning adenomas, an observation in concordance with many studies that have shown higher proliferation rates in these tumours (59, 80). In keeping with these findings, Scheithauer et al, found that although p27 was lower in carcinomas compared to invasive adenomas (59). Interestingly, Nakabayashi et al. found that p27 expression was lower in recurrent adenomas compared to non-recurrent ones (45). These results consistently show reduced expression of p27 and p21 are seen in pituitary adenomas compared to normal pituitary, in keeping with observations in other malignancies.

However, investigation of aberrations in p21 and p27 genes showed no mutations in one study (21) and in another study, no differences in p27 protein levels were detected in 18 pituitary tumours, 5 of which showed a polymorphism (codon 109, Val→Gly) (62). Correspondingly, Jin et al, found no differences in p27 mRNA expression between nontumourous, adenomatous, and metastatic pituitary tumours (24). Thus the lower expression of these cell cycle inhibitors may be due to post-translational mechanisms such as increased degradation (33). Investigating the expression of phosphorylated p27 (phospho-p27), the inactivated form of p27, Korbonits et al found that corticotroph adenomas exhibited higher levels than did other adenomas (33). The latter exhibited reduced phospho-p27 compared to normal pituitary whereas its levels were similar in normal pituitary and corticotroph tumours (33). The phospho-p27/p27 ratio was significantly higher in corticotroph adenomas, compared to metastatic tumours, invasive tumours, as well as TSH adenomas (33). On the other hand, in pituitary carcinomas both phospho-p27 and p27 levels were reduced. The variable phospho-p27/p27 ratio in pituitary tumours may show that the balance between the phosphorylated and unphosphorylated forms of
p27 protein may regulate tumour progression (33). Interestingly, earlier studies demonstrated reduced p27 levels in ACTH adenomas (2, 24, 80). Indeed, LH and TSH cell adenomas stained more frequently for p27; ACTH adenomas had the lowest levels of p27 protein in the study of Jin et al. (24). Similarly, Bamberger et al. showed that p27 negative cells occurred more often in corticotroph adenomas in contrast to gonadotroph adenomas in which p27 expression was higher than in other pituitary adenoma subtypes (2). Nonetheless, the significance and mechanisms underlying reduced p27 levels in pituitary tumours is unclear. Whether reduced p27 and/or p21 expression is a primary event in pituitary tumour initiation and progression or is secondary to other tumourigenic factors is unclear and requires further investigation.

**P21**

Similar to the above-mentioned studies that demonstrated reduced p27 expression in endocrinologically non-functioning tumours as compared to ones functional, significantly lower expression levels of p21, another inhibitor of cyclin-dependent kinases and a cell cycle blocker, were observed in non-functioning adenomas, as compared to functional adenomas (46). Whereas 71% of non-functioning adenomas showed p21 expression in less than 5% of cells, 77% of hormone secreting adenomas exhibited expression in more than 25%. Of note is that GH adenomas showed p21 expression in more than 75% of cells. Hiyama et al, also found lower levels of p21 in non-functioning as compared to secreting adenomas (17). These differences in p21 and p27 expression between non-functioning and secreting tumours may be a reflection of a fundamental difference in cell cycle regulation between these tumour types. They may even indicate that these two cell cycle inhibitors are involved in cellular differentiation (17).
Reduced expression of p27, a cell cycle inhibitor, is generally demonstrated in aggressive tumours in many studies (38, 40, 45, 62). In addition, nonfunctioning tumours tend to show reduced p27 as well as p21 expression as compared to functioning tumours, thus suggesting that these two cell cycle inhibitors play a role in the functional differentiation of pituitary tumours and may serve as markers distinguishing these two tumour classes (45, 62). Further studies of p27 with respect tumour behavior and subtype are much needed.

**P53**

P53 expression has been linked to aggressive tumour behavior. Thapar et al. demonstrated a significant association between tumour behavior and p53 expression, labeling of 0%, 15.2% and 100% being seen in non-invasive and invasive adenomas, as well as carcinomas, respectively (p<0.001) (63). Recently, Wierinckx et al. reported significantly higher p53 expression in “aggressive-invasive” tumours as compared to those with less aggressive behavior (p<0.0001) (74). Studying 41 pituitary tumours, Ozer et al. showed that elevated p53 expression was an independent indicator of local relapse (p=0.002), thus suggesting that p53 status is associated with tumour progression (51). In contrast to these reports, other studies of p53 levels and tumour recurrence, invasiveness, and/or volume (16, 59, 60) found no correlations, thus raising questions regarding the relevance of p53 expression as a marker of recurrence.

**Apoptosis**

Apoptosis, or programmed cell death, is defined by morphologic changes including cellular shrinkage and nuclear demarcation. Apoptosis is generally suppressed in neoplasms, thus disturbing the normal balance between mitotic and apoptotic activity and contributing to
increased tumour growth. Studies of apoptosis in pituitary tumours are limited in number. Vidal et al, examining over 8000 pituitary tumour biopsies, reported that most apoptotic activity was observed in corticotroph adenomas, only occasional examples being seen in PRL or gonadotroph adenomas (73). Several studies have investigated the relevance of apoptotic activity as a clinicopathologic marker (20, 35). Kontogeorgos et al noted higher apoptotic activity in aggressive, drug-resistant adenomas, indicating that apoptosis may be a useful prognostic marker (30). Similar findings were reported by Kulig et al (35) who observed a fourfold increase in apoptotic activity in pituitary carcinomas as compared to adenomas. Correspondingly, lower expression of bcl-2 anti-apoptotic factor, was seen in pituitary carcinomas as compared to adenomas and the non-tumoural pituitary gland (35). On the other hand, Ibrahim et al. found that apoptotic indices were not predictive of the growth rate of non-functioning pituitary tumours (20). These findings echo those of Nakabayashi et al, who in a series of 48 pituitary adenomas, found no significant difference between apoptotic indices in recurring and non-recurring adenomas (45). Lastly, in a series of 51 pituitary adenomas, Losa et al. found no significant difference in apoptotic index between ACTH macro- and microadenomas (41).

With respect to the functional status of pituitary tumour, Kontogoergos et al, (30) showed that hormone secreting adenomas had higher indices than did non-functioning tumours; highest apoptotic indices were observed in TSH adenomas, followed by GH, PRL, and mixed GH/PRL adenomas (30). Similarly. Sambaziotis et al. found that functioning adenomas exhibit higher apoptotic indices than ones non-functioning (58). In contrast, Green et al. found a higher apoptotic index in nonfunctioning tumours compared to GH adenomas (33% vs. 11%), a difference not statistically significant (15).
The expression of bcl-2 and bax, anti-apoptotic and pro-apototic factors respectively, also correlate with apoptotic indices. Interestingly, bcl-2/bax ratio has been found to be higher in non-functioning adenomas (58), highlighting the differences in the balance of anti- to pro-apoptotic activity in these tumours (58). Although these findings suggest that bcl-2/bax ratio is a useful marker of apoptosis and possibly of tumour behavior (51, 58), Ozer et al. found decreased expression of bcl-2 in non-functioning as well as in PRL-adenomas. It was concluded that downregulation of bax protein was associated with pituitary tumour progression (51). These discrepant findings may be due to evaluation of bcl-2 and bax expression rather than the bcl-2/bax ratio, which might be a better prognostic indicator.

With respect to apoptotic index and its correlation with clinicopathologic parameters results are highly variable and fail to support its utility as a prognostic indicator (20, 30, 35, 41, 51, 58). Similarly, although elevated p53 expression levels are shown to correlate with aggressive tumour behavior, not all studies confirm these findings (16, 32, 51, 59, 60, 61, 74). Thus, relevance of apoptotic index and p53 expression to tumour behavior is debatable.

Other markers

Galectin-3

Galectin-3 has been implicated in several biological processes including tumour progression, apoptosis, and metastasis (55). In a study of 162 pituitary tumours, including 14 carcinomas, Riss et al. found Galectin-3 to be expressed only in PRL and ACTH tumours, all other tumour types being immunonegative (55). In addition, Galectin-3 staining was found to be significantly higher in ACTH carcinomas compared to adenomas (55). This specific pattern of
Galectin-3 expression by PRL and ACTH tumours may be significant, since most pituitary carcinomas are mainly PRL- or ACTH-producing carcinomas (55). Furthermore higher Galectin-3 expression was found in functioning ACTH adenomas as compared to silent corticotroph adenomas (23, 65). As Galectin-3 appears to be a promising marker, validation studies to crystallize its role as a marker of pituitary tumour behavior are warranted.

Studies of Galectin-3 expression are limited in number, but show some promise (23, 55, 65). In as much as Galectin-3 is exclusively expressed in ACTH and PRL tumours, the most common form of pituitary carcinomas, further studies may establish a role for Galectin-3 in tumour differentiation and aggressiveness.

**Pituitary Tumour Transforming Gene (PTTG)**

Pituitary tumour transforming gene (PTTG) was first isolated from rat pituitary tumour cells (53). Since then, its expression in pituitary tumours has been demonstrated by many studies (8,10,18,42,43,57). Subsequent studies have identified PTTG as the human homolog of securin, a protein mediating sister chromatid separation during mitosis (81). Comparing PTTG mRNA expression in 54 pituitary tumours, Zhang et al et al showed no correlation with radiological tumour stage in clinically nonfunctioning adenomas, but did see significantly higher levels in hormone secreting, invasive tumours as compared to ones non-invasive (79). This 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 nonfunctioning tumours, the elevation being 2.7 fold (18). Although PTTG was also higher in these tumours as compared to PRL and ACTH adenomas, no statistical significance was reached (18).
Several studies have also investigated correlations between the expression of PTTG and other markers relevant to pituitary tumours particularly VEGF and bFGF, as PTTG promotes angiogenesis in many settings (42,43,44). 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 (42). Yet another study found many tumour cells to show PTTG co-localization with VEGF, as well as a high correlation between PTTG expression and the number of CD-34 positive blood vessels in GH-secreting pituitary adenomas (44). On balance, these various 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 tumours, Filippella et al., found a cut-off value of 3.3%, with 60% sensitivity and 76% specificity (10). However, there was no significant correlation between PTTG immunopositivity and tumour size or grade, patient age or gender, or tumour treatment (10). Where greater than one-year follow-up was available (27 of 45 patients), Ki-67 labeling proved to be a superior predictive marker of recurrence, the cut-off being 2.9% (10). Further studies are required to determine the importance of PTTG in pituitary tumour development.

Recent studies support the role of emerging technologies in finding new markers of the biology and behaviour of pituitary adenomas. For example, Ruebel et al, utilizing microarray analysis, demonstrated differential gene expression profiles for various pituitary adenoma subtypes and uncovered novel genes of possible value as predictive markers (56). Using microarray technology to analyze PRL tumours, Wierinckx et al found a set of diagnostic markers which included PTTG (74). The complexity of interactions between several molecular and cellular pathways in pituitary tumour 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 tumour behavior.

The most conclusive observation with respect to PTTG appears to be its upregulation of VEGF and FGF expression, both being overexpressed in various malignancies (42, 43, 44). Further studies into the role and prognostic value of PTTG in pituitary tumours are necessary.

**Conclusion**

Herein, we have explored various markers of pituitary tumours. We have also stressed the need for more consistent tumour definition and study criteria. What has become clear is that a wide variety of molecules affect pituitary tumourigenesis. Thus the need to develop a comprehensive number of markers, rather than reliance upon single ones has emerged. Newer techniques including DNA and microRNA microarrays will undoubtedly provide new candidates important to the development and progression of pituitary tumours.

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