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The pathology of pituitary adenomas from a clinical perspective

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1. ABSTRACT

Pituitary adenomas present with a variety of clinical endocrine manifestations and arise in a sporadic setting or rarely as part of hereditary genetic syndromes. Molecular analysis of familial pituitary adenomas has provided significant insight into pituitary tumorigenesis. Some specific genes have been identified that predispose to pituitary neoplasia, but these are rarely involved in the pathogenesis of sporadic tumors. The number of identified genes involved in pituitary tumorigenesis is progressively increasing. The possible resulting mechanisms of action involve abnormalities in signal transduction pathways, cell cycle regulators, growth factors, chromosome stability and others. Further studies are needed to evaluate the clinical significance of genetic alterations and their implications for patient prognosis, as well as to identify targets for existing and new therapeutic options. The aim of this review is to focus on the molecular pathology of pituitary adenomas from a practical perspective and discuss the possible clinical implications which may relate to particular molecular alterations. We have summarised familial syndromes related to pituitary adenomas and considered the prognostic value of selected molecular alterations in these tumors.

2. INTRODUCTION

Pituitary adenomas account for 10% to 25% of intracranial neoplasms (1) and are frequently seen in autopsy specimens (14%) (2). Epidemiological data suggest that clinically apparent pituitary adenomas have a prevalence of approximately one in 1,000 in the general population while radiological and autopsy assessment demonstrated that pituitary incidentalomas may be found in one in six people (3). These novel data show that the prevalence of clinically-relevant pituitary adenomas is 3-5 times higher than previously reported (4, 5). This high occurrence of pituitary adenomas adds impetus to research into the etiology of these tumors.

Pituitary tumors cause significant morbidity in affected patients, however, they can be managed effectively with long-term survival and they are very rarely malignant. Patients with pituitary tumors present with a variety of signs and symptoms. Some of the signs and symptoms are related to, or caused by, excessive hormone production, mass effects of an expanding tumor within the sella turcica (including headaches, visual disturbances and cranial nerve palsies) or impaired normal pituitary function (partial or panhypopituitarism). Approximately 50% of newly

diagnosed pituitary adenomas are prolactinomas (PRL-omas). Endocrinologically inactive adenomas – non-functioning pituitary adenomas (NFPAs) – represent about 30%, somatotroph adenomas (GH-omas) 15–20%, corticotroph adenomas (ACTH-omas) 5–10% and thyrotroph adenomas (TSH-omas) less than 1% (6, 7). True gonadotrophin-secreting pituitary adenomas resulting in clinical syndromes are extremely rare, but it seems likely that the great majority, if not all, NFPAs are in fact silent (non-secretory) or very-low-grade secreting FSH/LH-omas (8). Pituitary carcinomas comprise around 0.2% of pituitary adenomas (1), distinct from the 1% of pituitary mass lesions, which are metastatic tumours from non-pituitary sites. Advances in molecular biology, immunocytochemical staining and imaging, and the introduction of new treatment options, have all improved our understanding of the natural history of pituitary adenomas and their management. Available treatments include surgical, medical and radiation therapy.

It has been demonstrated that pituitary tumors are monoclonal in origin (9) arising from a single cell mutation, followed by clonal expansion. To understand carcinogenesis and tumor formation in man, animal models are often used. There is no good murine model of pituitary neoplasms. Despite the fact that naturally occurring pituitary tumors are common in rodents, their histologic origin (mostly from the intermediate lobe), age of presentation (late in murine life) and clinical course make them unsuitable models for their human counterparts (10). The genetic causes of common pituitary tumors remain for the most part unknown, especially in the context of sporadic pituitary adenomas.

In this article we will review the molecular biology of familial and sporadic pituitary adenomas, especially focusing on clinical aspects of these findings. We will also consider the practical utility of the knowledge coming from molecular biology for daily routine patient care, especially in the context of prognosis and recurrence. We do not aim to provide exhaustive information about all molecular abnormalities seen in these tumors, to which several recent reviews have been dedicated (7, 10-15). We would rather aim to emphasize the most important alterations that physicians dealing with pituitary adenomas should consider when seeing a patient with one of these tumors.

3. MOLECULAR ALTERATION IN FAMILIAL PITUITARY ADENOMAS

Pituitary tumors are rare in childhood and adolescence, with a reported prevalence of only one per million children. Only 2-6% of surgically-treated pituitary tumors occur in children. In children, more frequently than in adults, pituitary tumors may be a manifestation of a genetic condition (16), although even in adults pituitary adenomas may be a manifestation of an underlying germline syndrome; such disorders include McCune-Albright syndrome (MAS), multiple endocrine neoplasia type 1 (MEN1), Carney complex (CNC) and, most recently, the MEN1-like phenotype and pituitary adenoma predisposition (PAP) syndromes (10, 14, 17).

The genes for GNAS (at 20q13), menin (at 11q13) and protein kinase A regulatory subunit-1-alfa (PRKAR1A, at 17q24) have been associated with MAS, MEN1 and CNC, respectively. The cyclin-dependent kinase inhibitor 1B (CDKN1B, which codes for p27, at 12p13) and aryl hydrocarbon receptor (AHR) interacting protein (AIP, also at 11q13, but distinct from menin) are associated with an MEN1-like phenotype and PAP, respectively (18, 19). CDKN1B only accounts for a very small fraction of individuals with a MEN1-like syndrome and which are MEN1-gene mutation-negative (20). AIP is mainly mutated in the subset of families with familial isolated pituitary adenomas which have familial acromegaly (isolated familial somatotropinomas - IFS) and prolactinomas, (21-23), but even in this syndrome the majority of patients do not show a mutation in AIP (24, 25).

The McCune-Albright syndrome (MAS) is caused by mosaicism for a mutation in the *GNAS* oncogene. The gsp mutation (a mutation of the $G_s$alfa gene) causes constitutive activation of the cAMP pathway. MASis characterized by polyostotic fibrous dysplasia, pigmented skin lesions and over-activity of almost all endocrine glands, including the pituitary (26). Up to a fifth of MAS patients have GH excess but very few develop detectable pituitary adenomas (27). The *GNAS* mutation is the only mutation that has been identified in a significant proportion of sporadic pituitary tumors, occurring in approximately 30–40% of GH-omas. There is little direct evidence that the gsp mutation plays an essential primary oncogenic role, or that it alters tumor growth or recurrence rate, but it clearly is of tumorigenic significance (28, 29). Tumors with this mutation tend to be smaller and have higher GH secretion, and are most studies have suggested that they are more sensitive to dopamine- or somatostatin-induced GH inhibition (28).

Mixed PRL- and GH-secreting pituitary adenomas are relatively common because somatotrophs and lactotrophs share the common somato-mammotroph progenitor lineage. Recently, the evolution of an aggressive prolactinoma into a growth hormone-secreting pituitary tumor has been reported (30). In this report a patient with a prolactinoma was described who, after 15 yr of disease control by bromocriptine, became resistant to dopaminergic drugs. This patient underwent four neurosurgical procedures and two stereotactic radiotherapies and then unexpectedlydeveloped acromegaly. This patient's histology showed high Ki-67 and p53 and low D2 receptor expression. Samples from the initial surgery were positive for prolactin and negative for GH; however, about 10% of GH-positive cells were detected in tissue from the forth surgical intervention. Molecular screening did not find any mutations in RAS, TP53 or BRAF 'hot spots', whereas an Arg201His mutation in the GNAS gene (gsp oncogene), absent in the previous surgical material, was detected in the tumor from the last surgical procedure (30). This case report emphasises the observation that the appearance of a

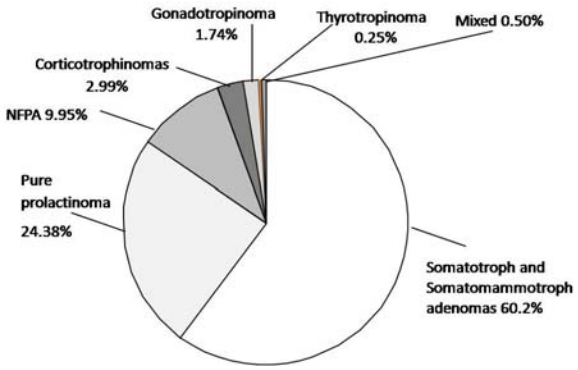

Figure 1. Pituitary tumour types in patients with familial isolated pituitary adenomas (25).

gsp oncogene in a prolactinoma evolving into acromegaly might confirm that this mutational change is associated with somatotroph growth and transformation (30).

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal-dominant familial disorder with a mutation to the *MEN1* gene (11q13) (31). The condition is characterized by predisposition to pituitary adenomas (25-40%), parathyroid hyperplasia (>90%), and pancreatic endocrine tumors (30-40%) (31). MEN1 germline mutations predispose to all major pituitary adenoma subtypes, with the most common subtypes of these secreting PRL (60%) and GH (20%), respectively. ACTH-secreting and non-functional adenomas represent 15% of MEN1 pituitary tumors (32). There is no phenotype-genotype correlation in MEN1 patients (33). In sporadic tumors allelic deletions on 11q13 are often observed (34), but somatic MEN1 mutations are very rare (35). In a very recent study which compared the biology of pituitary adenomas due to MEN1 syndrome with sporadic adenomas, MEN1-related tumors were significantly larger, more invasive and frequently pluri-hormonal then sporadic tumors, and MEN1 patients with large pituitary tumors (grade IV) were younger than non-MEN1 patients (33).

A normal MEN1 gene occurs in up to 10–25% of patients with MEN1 (36, 37). A germline nonsense mutation in the human cyclin-dependent kinase inhibitor 1B (*CDKN1B*, known also as p27/KIP1) gene was identified in a family with an MEN1-like condition (18); however, the occurrence of *CDKN1B* mutations is very rare in MEN1-like families (20). No mutations in the p27 gene have been found in sporadic pituitary tumors (38), but p27 protein remains under-expressed in most human pituitary adenomas, and is especially low in carcinomas (39-41). p27 is an important downstream signal in the menin signaling pathway, a possible target of oncogenic RET in endocrine cells (42), and also a direct transcriptional target of AHR (the partner of AIP, affecting cell proliferation) (43).

Carney complex (CNC) is an inherited neoplasia syndrome characterized by spotty skin pigmentation, myxomas, endocrine tumors and Schwannomas. The gene encoding for the protein kinase A (PKA) type 1A regulatory (R1alfa) subunit (PRKAR1A), like menin, acts as a tumor suppressor gene in affected tissues. Mutations in the PRKAR1A gene were found in 40% of CNC (44) and loss of its normal allele at chromosomal region 17q22–24 is present in pituitary tumors associated with CNC (45). A study performed on mice with pituitary-specific knockout of the PRKAR1A showed that its complete loss is sufficient to demonstrate abnormalities in the GH axis and eventually pituitary adenomas (46). Among the endocrine tumors that comprise the syndrome, GH-producing pituitary tumors are seen in approximately 10% of patients, although biochemical abnormalities of the GH axis are much more common, and somatotroph hyperplasia can be shown to precede adenoma formation. PKAR1A regulates the enzyme protein kinase A, which is activated by cAMP. In the absence of a normally-functioning regulatory subunit, the catalytic subunit becomes constitutively active and signals in the absence of cAMP, phosphorylating cyclic AMP response element binding protein (CREB) which acts as a nuclear transcription factor. However, no PKAR1A mutation has been demonstrated in sporadic pituitary tumors (47, 48).

Familial isolated pituitary adenoma (FIPA) is a recently recognized syndrome which embraces IFS and the PAP syndrome. Somatotropinomas or prolactinomas are the most common tumors found in FIPA; however, all types of pituitary adenomas can occur in this familial disorder. In FIPA patients there is a lower proportion of prolactinomas and higher frequency of somatotropinomas in comparison to MEN1 families. Patients with FIPA are significantly younger at diagnosis (21, 49) and have significantly larger pituitary adenomas than sporadic pituitary adenoma patients (13, 21, 22). PAP constitutes the subgroup of patients within FIPA who show germline mutations in AIP. The AIP protein molecule has three tetratricopeptide repeat motifs (TPRs) that are important sites for protein-protein interactions with the aryl hydrocarbon receptor (AHR), heat-shock proteins (HSP90), and many other proteins (25). AIP modulates the subcellular localization of AHR, which participates in cellular signaling pathways (50), including modulation of p27, while AIP also attenuates the activity of phosphodiesterase-4A5 (PDE4A5), which modulates cAMP signalling (Figure 1).

Recently, a multicenter, international, collaborative study was conducted focusing on the assessment of the frequency of AIP gene mutations in FIPA: AIP mutations were found in approximately 15% of affected families (22). In this study both affected members from each FIPA family as well as relatives of patients with AIP mutations underwent AIP sequence analysis. In 73 FIPA families (with 156 patients with pituitary adenomas), 11 FIPA families showed 10 germline AIP mutations; in AIP mutation-positive patients, the tumors were significantly larger and were diagnosed at a younger age, compared to mutation-negative subjects. In terms of tumor type, somatotropinomas predominated among families with AIP mutations, but mixed GH/prolactin-secreting tumors, prolactinomas and non-secreting adenomas were also reported (22).

AIP appears to be a tumor suppressor gene, since mutations to the AIP gene resulted in unchanged cell proliferation when inserted into GH3 pituitary cell lines,

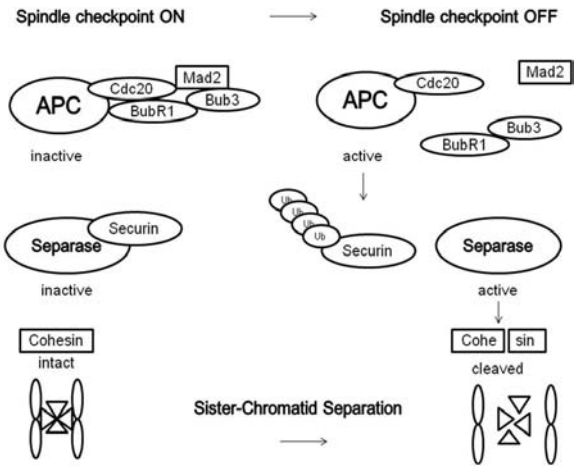

Figure 2. Molecular mechanism of chromosome segregation. At the metaphase–anaphase transition, APC/C^{Cdc20} ubiquitinates securin (PTTG). Degradation of securin activates separase. Separase then cleaves the Scc1 subunit of cohesin, allowing chromosome segregation. In response to sister-chromatids not being properly attached to the mitotic spindle, the spindle checkpoint promotes the assembly of checkpoint protein complexes that inhibit the activity of APC/C, leading to the stabilisation of securin, preservation of sister-chromatid cohesion, and a delay in the onset of anaphase (17). Reproduced with permission from Elsevier, License Number 2427120603871

HEK293 cell lines and TIG3 cell lines compared with markedly reduced cell proliferation in cell lines engineered to over-express AIP (21). However, the role of AIP mutations in pituitary adenoma development seems to be limited. Approximately 85% of the FIPA cohort and 50% of those with familial somatotropinomas were negative for AIP mutations (22). These germline mutations are extremely rare in sporadic pituitary adenomas, while somatic AIP mutations were not found in in sporadic tumors (21).

There have been a few case of pituitary adenomas (ACTH-omas and NFPAs) reported in patients with tuberous sclerosis (TS) patients); however the correlation between TSC gene alteration and development of pituitary adenomas remains uncertain (51).

4. SELECTED MOLECULAR ALTERATION IN SPORADIC PITUITARY ADENOMAS

Recent studies indicated multiple molecular alterations involving the cell cycle in pituitary adenomas. Many of them reflect epigenetic mechanisms associated with gene silencing, particularly of cell cycle inhibitors. Several novel genes had been isolated in pituitary adenomas including PTTG, Pdt-FGFR4, GADD45G, MEG3A, ZAC, DAP kinase or PTAG. Molecular alterations in tumour suppressor genes, oncogenes, growth factors and cell cycle regulators have been reviewed extensively in our previous review, including the newest results from RNA microarrays and proteomics of pituitary adenomas (17). Information related to deregulation of normal feedback processes in pituitary-specific signaling pathways have also been addressed previously (17).

Of interest, alterations in the best known tumor suppressor genes (e.g. *P53, RB, etc*) or oncogenes (e.g. the *Ras*-family) are rarely involved in pituitary adenoma development, even though they are very common in other neoplasms (52-55). However, point mutations of the *Ras* oncogene (53), loss of heterozygosity (LOH) near the *RB* locus on chromosome 13 (56, 57) and LOH on chromosome 11 (54, 55), have all been implicated in some pituitary tumors. A mutation of the *H-Ras* gene (codon 12, Gly to Val) was found in recurrent, highly invasive prolactinomas (53). *P53* gene mutations related to p53 protein over-expression in tumor cells have been reported in pituitary carcinomas, but not in pituitary adenomas (58).

The novel pituitary tumour transforming gene (PTTG) was identified and cloned in rat GH4 pituitary tumor cells (59). PTTG over-expression does not appear to be specific for pituitary adenomas since it occurs in a wide variety of endocrine and non-endocrine tumors which occur in the pituitary, thyroid, ovary, breast, prostate, lung, esophagus, colon, and the central nervous system (60). PTTG plays an important role in mitosis, acting as a securin protein and inhibiting premature sister chromatid separation (61). PTTG blocks sister chromatid separation during metaphase by binding to separin and preventing cohesin degradation (62) (Figure 2). Over-expression of PTTG *in vitro* induces cell transformation in NIH 3T3 fibroblasts and stimulates basic fibroblast growth factor (bFGF) (63), interacting with the *P53* tumor suppressor gene and *c-myc* proto-oncogene (60). It seems that PTTG phosphorylation occurs via phosphoinositol-3-kinase (PI3K) and MAPK cascades (64, 65). Even though PTTG is involved in processes controlling cell proliferation and division, specific mutation of PTTG has not been shown in sporadic pituitary adenomas (67, 68, 69).

Cell signalling abnormalities have been identified in pituitary tumors: in particular, Raf/MEK/ERK and PI3K/Akt/mTOR pathways were found to be over-expressed and/or over-activated (17, 66). These pathways share a common root including initial activation related to the tyrosine kinase receptor (Figure 3), and we have speculated that a change to these receptors or their relationship to membrane matrix-related proteins may be an early event in pituitary tumorigenesis.

PI3K is activated as a result of the ligand-dependent activation of tyrosine kinase receptors, G-protein-coupled receptors, or integrins (67, 68). The phosphorylation target of PI3K is Akt (also known as protein kinase B), and this can lead to the phosphorylation of a host of other proteins that affect cell growth, cell cycle entry, and cell survival. Akt phosphorylation activates a serine-threonine kinase mTOR (mammalian Target Of Rapamycin), which activates 40S ribosomal protein S6 kinase (p70S6K) (69), and inactivates 4E-binding protein (4E-BP1) (Figure 3).

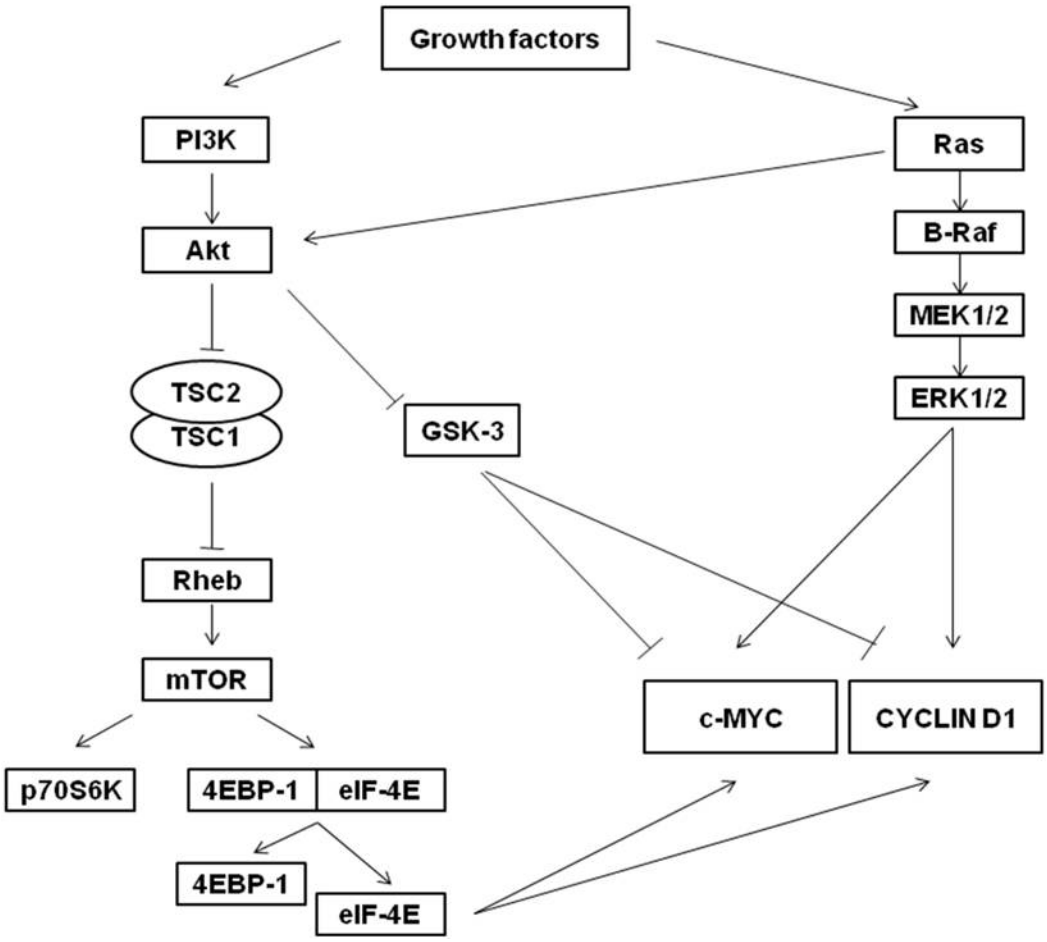

Figure 3. B-Raf/MEK/ERK and PI3K/Akt/mTOR pathways with their two main downstream effectors, c-MYC and CYCLIN D1. Interactions with other pathways such as the beta-catenin signaling system etc have been excluded for ease of illustration

Recent assessment of the genetic alterations in the PI3K gene showed that 8 out of 91 (9%) invasive pituitary tumors versus none out of 262 (0%) non-invasive tumors were found to harbor somatic mutations in exons 9 and 20 of the PI3K gene, and the mutation was associated with increased disease recurrence. In addition, genomic PI3K amplification (defined as >/=4 copies) were observed in both invasive and non-invasive tumors, with a prevalence of around 20-40% in various types of pituitary tumor (70).

Akt is over-expressed (at both mRNA and protein levels) as well as over-activated (through phosphorylation) in all pituitary tumors, especially NFPAs (71). This up-regulation of Akt will increase the phosphorylation of p27, preventing its nuclear import and influencing cell cycle regulation. Therefore, changes in cell-cycle occurring in pituitary adenomas may be secondary to activation of the Akt pathway (71).

The Raf/MEK/ERK signaling pathway is a hierarchical cascade originating at the cell membrane with receptors for mitogens or growth factors which recruit, via adapter proteins and exchange factors, the small guanosine triphosphatase (GTPase) Ras (72). Activated Ras in turn activates Raf, which is a serine-threonine kinase. Raf activates mitogen-activated protein kinase kinase (MAPKK), also known as MEK; MEK, in turn, phosphorylates and activates mitogen activated protein kinase (MAPK, or ERK1 and ERK2) (73) (Figure 3). The MAPK pathway activation causes phosphorylation and activation of ribosomal S6 kinase and transcription factors such as c-myc, Elk1, c-Fos and cyclin D1 (74, 75), similar to the the Akt/mTOR cascade, resulting in the activation of genes associated with proliferation (76) and leading to cell transformation (77). Over-activation of Akt may also lead, via changes in the kinase GSK3β, to modulation of the β-catenin pathway which has been implicated in pituitary tumorigenesis (11).

The most sensitive Raf, B-Raf, is frequently mutated at the V600E position in melanomas and papillary thyroid cancer leading to constitutive activity, but it is not similarly mutated in sporadic pituitary adenomas; however, it is over-expressed in pituitary adenomas, particularly NFPAs (78). Recently, RAS mutations were found in 6 out of the 91 (7%) invasive pituitary tumors, and these

mutations were mutually exclusive with PI3K gene alterations (70).

Data from our material indicates that MEK1/2 as well as its down-stream regulator ERK1/2 are over-phosphorylated and hence over-activated in all types of pituitary adenomas (PRL-, GH-, ACTH- and NFPA-omas), compared to the normal pituitary (66). Over-activation of ERK, an end-product of the MAPK pathway, was also associated with increased cyclin D1 (66), as previously reported (79, 80). Future studies should be focused on assessment of the role of consecutive ERK1/2 down-stream regulators and possible correlations between B-Raf/MEK/ERK and PI3K/Akt/mTOR pathways, especially as these two pathways have been shown to play an important role not only in malignant transformation but also in drug resistance (81) and might be a potential therapeutic target in pituitary adenomas (82).

5. PROGNOSTIC VALUE OF SELECTED MOLECULAR MARKERS IN PITUITARY ADENOMAS

Pituitary adenomas sometimes progress after surgery and can be locally invasive. Ki-67 and p53 expression are referred to as indicators of aggressive behavior in the World Health Organization Classification of Endocrine Tumors; however, the real value of these markers as indicators of tumor progression is controversial (83). In recent studies Ki-67 LI was found to be an independent predictor of progression (83) and recurrence in pituitary adenomas (84). In patients with adenomas with a Ki-67 LI of more than 3%, gross total removal was the most important prognostic factor; if this was not achieved, immediate adjuvant therapy was advised (85).

Assessment of apoptosis with the use of the TUNEL technique may provide some information in assessing the likelihood of recurrence of pituitary adenomas. Positive TUNEL labelling remains higher in recurrent cases when compared to non-recurrent cases (84), suggesting that a higher rate of proliferation, associated with a higher cell-cycle turnover rate, may cause a high rate of apoptosis (83).

As noted above, pituitary tumors are characterized by over-activity of both the Akt and the MAPK pathways and, while this is a finding in many tumor types, it suggests that cell cycle changes may be secondary to cell signaling abnormalities (17, 66, 86). Recently, a study concerning the utility of several factors including Akt/MAPK pathways for predicting the recurrence of NFPAs has been published (84). In this study, which included 35 NFPAs, tumors with a high level of expression of phospho-Akt, phospho-p44/42 MAPK, and PTTG1 were associated with early recurrence (84). Phospho-Akt expression remains higher in pituitary adenoma tissue than in normal pituitary tissue (71), and activation of Akt leads to decreased apoptosis by inhibiting pro-apoptotic activity of Bcl-2-associated death promoter in TSH-secreting pituitary tumors, confirming the important role of phosphatidylinositol 3-kinase-Akt pathway in the tumorigenesis of pituitary tumors (71, 84, 87). In addition, MAPK exerts dual actions, a proliferative signal when involved in PTTG1 expression and an anti-proliferative signal when involved in the dopamine-induced anti-proliferative effect and cell death in pituitary tumor cells. In pituitary cells containing the dopamine receptor (D2), dopamine exerts an anti-proliferative effect on p38, MAPK, and MEK1/ERK signaling (88). In our study activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways was found in all types of pituitary adenomas including GH-, ACTH-, PRL-omas as well as NFPAs (66). Translating molecular alterations to treatment, one mechanism for the therapeutic effect of octreotide is related to the inhibition of the MAPK signaling pathway after binding to the G protein-coupled somatostatin receptor. Non-phosphorylated Raf kinase inhibitory protein (RKIP) binds to and inhibits Raf-1 kinase, and thereby attenuates MAPK signaling, whereas phosphorylated RKIP inhibits G protein receptor internalization and degradation due to inhibition of G protein receptor kinase. Low levels of RKIP in growth hormone-secreting pituitary adenomas were found to correlate with poor response to octreotide treatment (89). The demonstration of over-activity of AKT and MAPK pathways qualifies them as valuable targets for inhibition mediated by somatostatin analogues (90). Targeting MAPK (Ras/ERK) and PI3K/Akt pathways in pituitary tumorigenesis has recently been reviewed, and it seems that these two pathways might be of particular interest in pituitary adenomas (82).

High levels of expression of phospho-CREB and ZAC1 in pituitary adenomas were found to be inversely associated with recurrence (84). CREB is a downstream signal of protein kinase A pathway activation by GHRH receptor in somatotropes (91, 92). The level of phospho-CREB expression is lower in non-functioning (non-GH-producing) adenomas than in functioning tumors (91). Expression of phospho-CREB could be a negative predictor of recurrence of NFPAs (84). The zinc-finger protein ZAC1 has a role as a transcription factor and co-regulator and also plays an important role in pituitary development, maturation and tumorigenesis. ZAC1 target genes control cell proliferation and hormone synthesis. Zac1 lies downstream to the mitogenic MAPK and survival PI3K pathways. Zac1 is an essential mediator of the anti-proliferative effects of this treatment and correlates to successful outcome in acromegalic patients (93). ZAC1, a tumor suppressor gene, is highly expressed in the normal anterior pituitary gland but is down-regulated in most pituitary adenomas. Inhibition of the PI3K/Akt signalling pathway by octreotide in pituitary tumor cells was associated with increased expression of the ZAC1 gene (94). Reduced expression of ZAC1 mRNA was reported in NFPAs and was not related to ZAC gene mutation (95). Furthermore, ZAC1 expression was completely absent in about one third of recurrent NFPAs, and its expression was weak in the remaining recurrent NFPA cases (84). Based on these recent resultsit is be possible that tumors with high levels of expression of phospho-Akt and phospho-p44/42MAPK, and low levels of expression of phospho-CREB and ZAC1, should be followed closely and may require adjunctive therapy to prevent tumor recurrence (84).

In pituitary adenomas, there are also changes in the β-catenin pathway, which is involved in cell contact inhibition through the cadherins. These changes at the level of the cell surface growth factor receptors, and their modulation by integrins and cadherins may hold the key to pituitary pathogenesis (11). Appropriate cell-to-cell adhesion is fundamental for the epithelial phenotype of pituitary cells and loss of the adhesion protein E-cadherin has been associated with invasiveness, metastasis, and poor prognosis in cancers of epithelial origin. In somatotroph adenomas, a variable and reduced expression of E-cadherin has been demonstrated and nuclear translocation of E-cadherin was found to correlate with pituitary tumor size and invasiveness, and responsivity to somatostatin analogue therapy (96).

In 207 fresh pituitary adenoma specimens, the usefulness of DNA-flow cytometry, (which provide an estimation of a tumor's proliferative rate) has been assessed. DNA-flow cytometry was performed to evaluate its capability to both assess prognosis and predict recurrence, and was helpful in identifying patients requiring closer follow-up, such as those with invasive adenomas and Nelson's syndrome. However, no single parameter revealed by DNA-flow cytometry could predict tumor prognosis or recurrence in a mean follow-up of 7.5 years (97).

Changes in PTTG expression may relate to pituitary tumor invasiveness and aggressiveness (60, 63, 98). Importantly, in pituitary adenomas, PTTG expression correlates with Ki-67 (70) and VEGF mRNA expression (99, 100). PTTG expression was found in NFPAs and in GH-secreting adenomas, but not in normal pituitary tissue (based on *in situ* hybridization) (63). Immunohistochemistry showed positive expression of PTTG in 90% of different types of pituitary tumors and confirmed lack of PTTG expression in normal pituitary tissue (98). RT-PCR showed that PTTG expression was present in normal pituitaries, but at a much lower level than in pituitary adenomas. PTTG was mostly over-expressed in adenomas with a >50% increase in NFPAs, GH-, PRL- and ACTH-omas (63). Over-expression of PTTG correlated with tumor invasiveness in hormone-secreting tumors. Higher PTTG expression was observed in tumors that had invaded the sphenoid bone (stages III and IV; 95% CI 3.118-9.715) compared with tumors that were confined to the pituitary fossa (stages I and II; 95% CI 1.681-3.051) (63). An increasing body of evidence suggests clinically significant implications of PTTG1 through its correlation with aggressive phenotypes or survival rate, and thus PTTG1 is an interesting candidate biomarker for malignancy, tumor staging and subsequent need for therapeutic interventions (90).

Fibroblast growth factors (FGFs) regulate mitogenesis, differentiation, development, angiogenesis and tumorigenesis (101), and mediate their biological effects by binding to high-affinity tyrosine-kinase receptors - the fibroblast growth factor receptors (FGFRs) (102). FGF-2 (also known as basic or bFGF) is over-expressed by pituitary tumor cells with higher levels in more aggressive tumors (103). Cytoplasmic expression of ptd-FGFR4 (the pituitary tumor-derived FGFR-4 isoform) was found in 60% of pituitary adenomas (GH-, ACTH-, FSH/LH- and NFPAs) but was relatively rare in PRL-omas and absent in normal tissue. Expression of ptd-FGFR4 was stronger in macroadenomas in comparison to microadenomas, and correlated with Ki-67 immunostaining (104).

Assessment of molecular markers seems to give additional information for prognosis in PRL-omas (105). In a retrospective study on 94 surgically-treated patients with PRL-omas, seven genes (ADAMTS6, CRMP1, PTTG, ASK, CCNB1, AURKB, and CENPE) were associated with tumor recurrence or progression, and five of these (ADAMTS6, CRMP1, ASK, CCNB1, and CENPE) were associated with the pathological classification (105).

6. SUMMARY AND PERSPECTIVE

There is some practical information which follows on from these studies which could be reasonably emphasized: 1. PRL-omas occur in 20% of MEN1 patients, but 60% of pituitary adenomas seen in MEN1 are PRL-omas. These PRL-omas seen in MEN1 patients might be more aggressive. Malignant PRL-omas are very rare and might be related to a *RAS* mutation, 2. GH-secreting tumors can be seen in the MEN1 syndrome (where they account for 25% of pituitary adenomas in MEN1 patients) and CC (less than 10% of cases), as well as IFS. Gsalfa mutations related with MAS were found in up to 40% of GH-secreting tumours. 3. In ACTH-omas, Gsalfa mutations were found in a minority of cases. RB gene mutations in mice lead to intermediate lobe ACTH-omas, but no mutations have been demonstrated in human pituitary adenomas, 4. NFPAs are the commonest pituitary macroadenomas, representing 25% of all pituitary tumors, but in NFPAs $G_s$alfa mutations were only found in a minority of cases.

Our understanding of these genetic conditions has expanded rapidly due to the identification of new predisposing genes including MEN1, MEN4, PRKAR1A, CDKN1B and AIP, but there is little information that the mutations occurring in genetic syndromes are common in sporadic tumors. Several genes (e.g.: PTTG, Pdt-FGFR4, ZAC, p27, PTTG) have been implicated in pituitary tumorigenesis. Cell signaling abnormalities have been identified in pituitary tumors and both the PI3K/Akt/mTOR pathway and the Raf/MEK/ERK pathway are over-expressed and/or over-active in many pituitary tumours. Some of molecular alterations seem to be related to tumor aggressiveness, invasiveness or recurrence, and may influence response to somatostatin analogues. However, for the vast majority of pituitary tumors the molecular defect has yet to be elucidated, and more genes for susceptibility are likely to be identified.

Future work should focus on understanding the molecular mechanisms that control pituitary tumor transformation, where intracellular signaling molecules will constitute not only diagnostic/prognostic markers but also novel therapeutic targets.

7. REFERENCES

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