MANAGEMENT OF ENDOCRINE DISEASE

Clinicopathological classification and molecular markers of pituitary tumours for personalized therapeutic strategies

Gerald Raverot^{1,2,3,†}, Emmanuel Jouanneau^{1,2,4} and Jacqueline Trouillas^{1,2,5}

¹INSERM U1028, CNRS UMR5292, Lyon Neuroscience Research Center, Neuro-Oncology and Neuro-Inflammation Team, Lyon F-69372, France, ²Université de Lyon, Université Lyon 1, Lyon F-69372, France, ³Fédération d'Endocrinologie, ⁴Service de Neurochirurgie and ⁵Centre de Pathologie Est, Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon F-69372, France

[†]G Raverot is now at Fédération d'Endocrinologie du Pôle Est, Groupement Hospitalier Est, Aile A1, 59 Bd Pinel, 69677 Bron Cedex, France

Correspondence
should be addressed
to G Raverot

Email
gerald.raverot@chu-lyon.fr

Abstract

Pituitary tumours, the most frequent intracranial tumour, are historically considered benign. However, various pieces of clinical evidence and recent advances in pathological and molecular analyses suggest the need to consider these tumours as more than an endocrinological disease, despite the low incidence of metastasis. Recently, we proposed a new prognostic clinicopathological classification of these pituitary tumours, according to the tumour size (micro, macro and giant), type (prolactin, GH, FSH/LH, ACTH and TSH) and grade (grade 1a, non-invasive; 1b, non-invasive and proliferative; 2a, invasive; 2b, invasive and proliferative and 3, metastatic). In addition to this classification, numerous molecular prognostic markers have been identified, allowing a better characterisation of tumour behaviour and prognosis. Moreover, clinical and preclinical studies have demonstrated that pituitary tumours could be treated by some chemotherapeutic drugs or new targeted therapies. Our improved classification of these tumours should now allow the identification of prognosis markers and help the clinician to propose personalised therapies to selected patients presenting tumours with a high risk of recurrence.

*European Journal of
Endocrinology*
(2014) **170**, R121–R132

Introduction

Pituitary tumours are the most frequent intracranial neoplasm, affecting 1/1000 of the worldwide population (1, 2). These tumours were once considered as benign; yet recent evidence suggests that while metastasis is rare, such a benign status needs revision. Besides, the endocrine signs

due to hormonal hypersecretion or pituitary deficit, pituitary tumour growth or invasion represent a greater clinical and therapeutic challenge. Indeed, 30–45% of pituitary tumours invade the cavernous or sphenoid sinus (3, 4) and a significant number are considered as aggressive based on

Invited Author's profile

Gérald Raverot is an endocrinologist at Lyon University Hospital and Professor of Endocrinology at Lyon1 University, France. He is joint in-charge (with Prof Emmanuel Jouanneau) of the Pituitary Center, Lyon. He leads a research team dedicated to "Pathophysiology of pituitary tumour" at the INSERM Research Center U1028 in collaboration with Prof Jacqueline Trouillas. His major research interests are pituitary tumour pathogenesis and identification of markers of aggressiveness allowing the identification of potential new therapies.

their resistance to conventional treatment or recurrence during follow-up (5, 6). Some rare aggressive tumours that develop metastasis are considered as carcinomas (7, 8, 9) and cannot be controlled by any available treatment (5, 8, 9, 10, 11, 12). The present-day clinical definition of aggressiveness does not allow the use of preventive treatment. The absence of consensual prognostic markers or a prognostic classification limits the evaluation of medical strategies for pituitary tumours. Different pathological markers have been suggested; however, their prognostic value has not always been confirmed. Although in 2004 the World Health Organization (WHO) classification proposed the term 'atypical tumour' with 'uncertain malignancy' (13), its definition is subjected to individual interpretation in routine practice and the prognostic value of this classification has never been evaluated. Taking these limitations into account, we recently proposed and evaluated a new clinicopathological classification of pituitary tumours (14).

Herein, we present a critical review of the pathological classifications of pituitary tumours to date and discuss the recent advances in the identification of pathological, molecular and genetic markers associated with tumour behaviour (not tumourigenesis), allowing a personalized management of patients with these tumours.

Pituitary tumour classifications

From the tinctorial classification to the 2004 WHO classification, endocrine pituitary tumours arising from adenohypophyseal cells are clinically classified into functioning (mainly growth hormone (GH) with acromegaly, prolactin (PRL) with amenorrhoea–galactorrhoea; adrenocorticotrophic hormone (ACTH) with Cushing's disease) and non-functioning (mainly follicle-stimulating hormone (FSH)–luteinising hormone (LH)) tumours. Over the years, technological progress and knowledge increase have driven an evolving pathological classification of pituitary endocrine tumours, from a tinctorial classification to an immunocytochemical classification (Table 1), and a new 'atypical' adenoma with uncertain malignancy (13). The diagnosis of carcinoma remains restricted to tumours with systemic metastasis.

Until the 1980s, pituitary tumours were classified based on their tinctorial properties with haematoxylin–eosin/phloxine safran and were associated with a clinical disease ineosinophilic or acidophilic (with acromegaly), basophilic (with Cushing's disease) and chromophobic adenomas. Later, with the development of electron microscopy and immunocytochemistry, the tumours were classified based on the appearance of their organelles

Table 1 Immunocytochemical classification of endocrine pituitary tumours.

Tumour types	Immunoprofiles
PRL tumour	
Densely granulated ^a	PRL
Sparsely granulated	PRL, α -SU
GH tumour	
Monohormonal	
Densely granulated	GH, (α -SU), Ck, (CgA)
Sparsely granulated	GH, (α -SU), Ck, (CgA)
Plurihormonal	
Mixed GH–PRL	GH, PRL, (α -SU, β -TSH)
Mammotroph	GH, PRL, (α -SU, β -TSH)
ACTH tumour	
Densely granulated	ACTH, β -endorphin, β -LPH, Ck, (CgA)
Sparsely granulated	ACTH, β -endorphin, β -LPH, Ck, (CgA)
TSH tumour	
Monohormonal ^a	β -TSH, α -SU
Plurihormonal	β -TSH, α -SU, GH, PRL
FSH–LH tumour	β -FSH, β -LH, (α -SU), CgA
Non-immunoreactive tumour ^a	(CgA)
'Silent' tumours	
Mono/plurihormonal	
ACTH	ACTH, β -endorphin, β -LPH
GH	GH, PRL (β -TSH)
TSH	β -TSH, α -SU, GH, PRL

^aRare subtype.

(granulations, mitochondria) and their hormonal secretion (15). In 1996, Kovacs *et al.* (16) proposed the WHO classification of adenohypophysial neoplasms using a five-tier scheme on the basis of functional, imaging/surgical, histological, immunohistochemical (IHC) and ultrastructural findings. Although this detailed classification was very interesting, it is highly complex and can only be used by specialized pathologists.

Nowadays, electron microscopy, an expensive and time-consuming technique, is only rarely carried out by some specialists, and the powerful IHC technique is used instead on a routine basis. Tumours are currently classified into five main IHC types – PRL, GH, ACTH, TSH and FSH–LH – which can be monohormonal or plurihormonal, with or without (silent) signs of hypersecretion (Table 1). Almost all the ultrastructural subtypes were found to be only morphological variants identifiable by IHC, and some disappeared with the improvement of the IHC technique (automation and new antibodies). The null cell adenoma (17) corresponds to FSH/LH tumours with low intracellular hormonal content, undetected by some antibodies. We prefer the term of non-immunoreactive tumours, which are now very rare (from 10% in 1992 to 1% in 2012 in Lyon's pathological series). The oncocytoma (18, 19) is a FSH–LH tumour type with numerous

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mitochondria. The ultrastructurally densely and sparsely granulated GH tumour is most easily identified by juxta-nuclear dots of cytokeratin, known as 'fibrous bodies', characteristic of this latter subtype. The cytokeratin expression is also very helpful in identifying ACTH tumours, which are usually strongly positive, though can have a weak staining in 'silent' ACTH tumours, which are also positive with galectin 3 (20). The transcription factors involved in pituitary cell differentiation immunostaining may be useful to confirm the diagnosis, particularly of non-immunoreactive or silent tumours (21). *PIT1* (*POU1F1*) is expressed in GH, PRL and TSH tumours (22); *T-PIT* in ACTH tumours with and without Cushing's disease (23) and *SF1* in FSH/LH tumours (24).

Silent corticotroph subtypes 1 and 2 (25) are clinical or cytological variants of ACTH tumours. As recently reported (26, 27), a number of patients with a 'silent' corticotroph tumour may present clinical symptoms of Cushing's disease at some stage during the tumour evolution; evidence also exists of a continuum from the macroadenoma with Cushing's to the 'silent' ACTH tumour (27). We therefore propose renaming such 'silent' tumours as 'ACTH tumour without signs of Cushing's', similar to the renaming of 'silent' GH tumours (28) as 'GH adenoma without acromegaly' (29). Acidophilic stem cell adenomas (25, 30) are either GH-PRL or PRL tumours with giant and dilated mitochondria. It seemed necessary to us to provide these precisions to clarify the present day terminology, because some of these subtypes, i.e. the sparsely granulated GH subtype (31), the silent ACTH (32), silent GH-PRL or subtype 3 adenomas (28), may be considered to be poorly differentiated tumours with potentially aggressive behaviour. They must be taken into account for post-operative management, especially in cases of residual tumour.

Moreover, the detection of somatostatin receptor types 2 and 5 could be helpful. GH and TSH tumours with low SST2R expression shown to represent a higher risk of resistance to octreotide/lanreotide, whereas ACTH tumours with high SST5R expression may respond better to pasireotide treatment. This detection is always negative in PRL tumours (33).

The 2004 WHO classification (13) classified all benign tumours as typical adenomas (ICD-0 8272/0), while atypical adenomas (ICD-0 8272/1) included all tumours showing 'borderline or uncertain behaviour'. Such tumours were classified as having atypical morphologic features suggestive of aggressive behaviour such as invasive growth. Other features include an elevated mitotic index and a Ki-67 labelling index >3%, as well

as extensive nuclear staining for p53 immunoreactivity. The pituitary carcinomas (ICD-0 8272/2) are rare tumours (0.2%), defined by the presence of systemic or cerebro-spinal metastases (9, 34). Although markers of proliferation, p53 detection, and invasion were all mentioned as the criteria of the 'atypical adenoma', the invasion has not been systematically taken into account, as illustrated by the two papers which have studied this type of tumour. In the Law's series of 121 consecutive patients (3), the frequency of atypical adenoma was 15%, compared with 2.5% in the Saeger *et al.*'s (35) series of 241 tumours from the German registry. This discrepancy is explained by the differences in criteria taken into account for the diagnosis: a Ki-67 >3%, p53 positivity and increased mitotic activity without a cut-off value in the Law's series (3), and invasion associated with Ki-67 >3% and P53 >5% in the Saeger series (35). Thus, as advised by Wolfsberger & Knosp (36), 'the definition of invasiveness is needed and should be included in this classification as in a previous classification' (37), and the proliferation must be evaluated by markers of the cell cycle with well-defined thresholds. Moreover, as some experts have pointed out (38, 39, 40), this classification needs to be correlated to clinical evolution, with post-operative results, progression and recurrence.

A prognostic clinicopathological classification

Recently, we have proposed a new clinicopathological classification (14), which takes into account both tumour size and the five IHC subtypes (PRL, GH, FSH/LH, ACTH and TSH). We set up a grading system, such as that used for other endocrine tumours of the foregut (41), based on invasion and proliferation status (Table 2). Magnetic resonance imaging (MRI) is needed to evaluate the invasion because the histological proof of invasion is rare (9% of invasive tumours). Only invasion into the sphenoid sinus, confirmed by the infiltrated respiratory mucosae on histology, and unequivocal invasion of the cavernous sinus were considered (42). Indeed, recent anatomical studies have shown that the medial wall of the cavernous sinus is composed of dura (43), which can be assessed preoperatively by an endoscopic technique. Using multivariate statistical analysis and a receiver operating characteristic (ROC) curve (14), we found that invasion is a major prognostic factor in predicting both the disease-free status, following the surgical removal of pituitary tumours (36), and recurrence/progression (44). In our opinion, invasion must be added to the synoptic checklist for pituitary lesions recently published by a group of experts (45), especially considering that the data

Table 2 Prognostic clinicopathological classification of the pituitary tumours.

The classification is based on the three following characteristics:

1. Tumour size into micro (< 10 mm), macro (≥ 10 mm) and giant (> 40 mm) by MRI
2. Tumour type into GH, PRL, ACTH, FSH/LH and TSH by immunocytochemistry
3. Tumour grade based on the following criteria:
Invasion defined as histological and/or radiological (MRI) signs of cavernous or sphenoid sinus invasion
Proliferation considered on the presence of at least two of the three criteria:
Mitoses, $n > 2/10$ HPF;
Ki-67, $\geq 3\%$ and
P53, positive (greater than ten strongly positive nuclei/ten HPF)

The five grades are the following:

- Grade 1a, non-invasive tumour
- Grade 1b, non-invasive and proliferative tumour
- Grade 2a, invasive tumour
- Grade 2b, invasive and proliferative tumour
- Grade 3, metastatic tumour (cerebrospinal or systemic metastases)

from pituitary imaging, and surgical findings are included in the patient database, which is now available to pathologists.

Tumour proliferation is evaluated by the two most commonly used cell-cycle markers in oncology (Ki-67 index and mitotic count) and p53. Considering the controversial value of these markers, especially Ki-67 (44, 46, 47, 48, 49, 50), and the lack of methodological standards and validated cut-off for p53 (50, 51), we defined proliferation as the presence of at least two of these markers with specified cut-off values. These were 3% for Ki-67 index and the number of mitoses being $n > 2/10$ high-power fields, as for endocrine pancreatic tumours (41). We also considered p53 in terms of its positivity rather than its precise percentage.

Our proposed classification displayed highly significant prognostic value for predicting post-operative disease-free outcome or recurrence/progression status across all tumours and for each type of tumour. At 8-year follow-up, the probability of a patient showing evidence of disease or tumour progression was 25- or 12-fold higher, respectively, if they had an invasive and proliferative tumour (grade 2b) as compared with if they had a non-invasive and non-proliferative tumour (grade 1a). These results confirm those obtained in our preliminary study on 94 PRL tumours (52).

Pituitary carcinoma, defined as a pituitary tumour with metastases, is rare and accounts for about 0.2% of pituitary tumours, with only 132 cases reported in the

literature from 1961 to 2009 (8). Most pituitary carcinomas are secretory, secreting PRL (36% cases) or ACTH (30% cases) in the majority of cases. Non-functioning tumours are less frequent (23% cases). Previously, histological signs of malignancy were thought to be absent, however, based on the frequency of certain signs in previous pathological series of pituitary carcinomas (34, 53), a potential malignancy in pituitary tumours could reasonably be suspected based on the association of the following pathological signs: invasion, neoangiogenesis, vascular invasion, abnormal mitoses, very high index of Ki-67 $> 10\%$, and p53 $> 5\%$ and genomic alteration (chromosome 11 for PRL tumours), which when combined might be considered as criteria of malignancy. However, these cut-off values were not validated and some of the above signs were found to be absent in certain metastatic pituitary carcinomas (53, 54). The observation that six out of the eight carcinomas of our series were grade 2b at the first surgery (14), together with the comparison of human PRL tumours with our animal model (SMtTW model) (55), led us to postulate that grade 2b or aggressive-invasive tumours are in fact malignant tumours without metastasis (56, 57, 58).

Molecular markers associated with tumour behaviour

Biological markers detected by immunohistochemistry

Although the expression of several biological markers has been investigated by IHC and correlated with invasiveness and/or aggressive behaviour, no single marker has been found to predict the tumour behaviour and until now their detection is only carried out on a research level. As two recent and exhaustive reviews have been published on this subject (21, 59), here we will focus on biomarkers that appear to correlate with invasive and aggressive behaviour of pituitary tumours.

Of the fibroblast growth factors (FGFs) and their receptors (FGFRs), which regulate growth, differentiation, migration and angiogenesis, FGF2 and FGF4 are expressed in the pituitary. Tumours show a loss of FGFR2, with a resultant up-regulation of MAGEA3 (60), and a truncated isoform of FGFR4, known as pituitary tumour-derived FGFR4 (pdt-FGFR4). The expression of the latter induces invasive growth of pituitary tumour cells *in vivo* with loss of membranous N-cadherin expression (61). Moreover, pdt-FGFR4 cross talks with a polysialylated form of NCAM, which correlates with invasiveness (62). The expression of endocan, a proteoglycan secreted by endothelial cells, has been found to be associated with size and progression of

pituitary tumours (63). The expression levels of matrix metalloproteinase 9 (MMP9) and the pituitary tumour-transforming gene (PTTG), which is a member of the securin family, are significantly higher in invasive pituitary adenomas (64, 65).

Gene expression by transcriptome analyses

Initial transcriptome studies compared normal pituitary with tumour as a whole or according to subtype. These studies provided interesting data concerning pituitary pathogenesis, but did not allow the identification of molecular markers associated with tumour behaviour or prognosis (66, 67, 68, 69). Recently, Wierinckx *et al.* (57) conducted a meta-analysis on these published results and identified only six genes (*GADD45B*, *SAT1*, *ID1*, *VIM*, *IGFBP5* and *ZFP36L1*), with known involvement in cell proliferation or tumorigenesis, that were shown to be down-regulated in most studies collectively comparing tumour and normal pituitary, and in at least one study comparing each tumour subtype with normal tissue.

With the exception of *GADD45 γ* (*GADD45G*), characterised by Zhang *et al.* (70), the functional involvement of the majority of these genes in pituitary tumour pathogenesis remains to be determined. Indeed, *GADD45* (*GADD45A*) expression has been extensively studied by independent groups (70, 71, 72, 73); all of which demonstrated that the down-regulation of *GADD45 γ* is more frequently found in non-functioning pituitary adenomas (NFPAs) than in functioning tumours (90 and 58% respectively) ((74, 75) for review), and that promoter methylation seems to be the major cause of loss of *GADD45* expression in pituitary tumours (71).

Using cDNA-representational difference analysis, Zhang *et al.* (76) identified a novel non-coding RNA gene, named maternally expressed gene 3 (*MEG3*), that is down-regulated exclusively in gonadotroph tumours. *MEG3* loss of expression is due to methylation within the functional regulatory regions of the *MEG3* gene and not due to its mutation (77). Functional data suggested that *MEG3* acts as a tumour suppressor, interacting with both p53 and Rb to control cell proliferation (78).

All these results comparing tumour to normal pituitary should be interpreted with caution because the pituitary comprises a mix of different cell types, whereas the tumours comprise an expanded population of one major cell type. Moreover, although these genes may be associated with pituitary tumorigenesis, none have been associated with tumour prognosis (79). To address this and attempt to find predictive factors of prognosis or

behaviour, recent studies have analysed the differential expression of gene according to the tumour characteristics. This approach allowed the identification of genes associated with tumour invasion (56, 80) or tumour aggressiveness (52, 56, 57, 81). Using microarrays to analyse the gene expression profiles of 40 NFPAs (22 invasive and 18 non-invasive), Galland *et al.* (80) identified four genes (*IGFBP5*, *MYO5A*, *FLT3* and *NFE2L1*) that were overexpressed. At the protein level, however, only *MYO5A* immunostaining was stronger in invasive than in non-invasive NFPAs. Recently, Marko *et al.* (81) adopted a more stringent strategy to select a group of NFPAs to study the gene expression profile of early recurrent compared with non-recurrent tumours. Using these stringent criteria and despite the limited number of cases ($n=11$), the authors were able to identify five genes that were differentially expressed but underlined in particular the potential role of *CHL1*, which encodes an extracellular matrix and cell adhesion protein involved in nervous system development (82). However, the short-term follow-up (mean 3.5 ± 0.81 years) of the non-recurrent group limited the value of their results, because a significant number of the NFPAs recurred between 5 and 10 years after surgery (83, 84). This study underscored the practical challenges associated with this type of analysis in pituitary tumours due to the limited access to tumours with clear clinical phenotype and long-term follow-up.

Following a similar stringent strategy, Wierinckx *et al.* (56) analysed the gene expression profiles of ten PRL-secreting tumours classified as non-invasive, invasive or aggressive-invasive tumours according to the clinical and pathological criteria (14), and identified a set of nine genes associated with the tumour classification. Among these nine genes, three were associated with invasion (*ADAMTS6*, *CRMP1* and *DCAMKL3* (*DCLK3*)), five with proliferation (*PTTG* (*PTTG1*), *ASK* (*DBF4*), *CCNB1*, *AURKB* and *CENPE*) and one with pituitary differentiation (*PITX1*). The identification of this set of genes was facilitated by the comparative study conducted in rat models of three different malignant PRL tumour lineages (55, 85, 86). It is interesting to note that this unsupervised analysis allowed the identification of *PTTG*, a well-known prognostic marker of pituitary tumours (65, 87).

Following this initial study, the prognostic values of these nine genes were evaluated in an independent cohort of 29 PRL-secreting tumours. Overexpression of seven out of the nine genes (*ADAMTS6*, *CRMP1*, *PTTG*, *ASK*, *CCNB1*, *AURKB* and *CENPE*) was associated with a higher risk of recurrence or progression during a long-term follow-up of more than 10 years (52).

Chromosomal alteration by comparative genomic hybridisation studies

Chromosomal abnormalities in general and more specifically copy number variation have been studied by numerous groups, which have revealed a high level of aneuploidy within these tumours. Bates *et al.* (88) studied loss of heterozygosity (LOH) associated with an aggressive behaviour and identified a significantly higher frequency of LOH in invasive tumours compared with non-invasive tumours and demonstrated that allelic deletion in the invasive tumours is clustered at four loci: 11q13 (multiple endocrine neoplasm type 1 (*MEN1*) and aryl hydrocarbon receptor-interacting protein (*AIP*)), 13q12–14, 10q26 and 1p. The frequency of these alterations has been confirmed in other publications (58, 89, 90, 91, 92, 93, 94). To identify the specific alterations associated with tumour behaviour and evaluate the impact of genomic alteration on transcriptomic activity, we recently conducted an integrated genomic profiling study of PRL tumours classified according to our pathological classification (58). The six aggressive PRL tumours of grade 2b (including three carcinomas) were compared with seven non-aggressive PRL tumours of grades 1a and 2a. We demonstrated that the 11p region was commonly deleted in the aggressive tumours and that this deletion had an impact upon the transcriptomic activity. In particular, the five genes located in this region (*CD44*, *TSG101*, *DGKZ*, *HTATIP2* and *GTF2H1*), related to the molecular pathway associated with PRL tumour aggressiveness, were down-regulated (52, 56). Moreover, the loss of the 11q arm and a gain of 1q arm were also observed in the three aggressive tumours that developed metastasis during follow-up and so became malignant (58). These results validated the biological value of our classification and suggested that the accumulation of new genomic alterations (11q loss and 1q gain) may transform an aggressive pituitary tumour into a pituitary carcinoma. Larger studies are, however, needed to confirm this hypothesis but are warranted because the identification of such markers would help to determine the need for additional treatment (e.g. radiotherapy) after surgery.

Epigenetic regulation by microRNA expression studies

MicroRNAs (miRNAs) are a class of small non-coding RNAs that are deregulated in many types of cancer. They act as tumour suppressors or oncogenes, depending on the function of the targeted genes, and seem to be involved in the regulation of many steps of tumour progression. Results

from studies comparing pituitary tumours to normal pituitary (95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108) suggest that miRNA deregulation may play a key role in pituitary tumourigenesis. Indeed, D'Angelo *et al.* (101) identified a set of miRNAs, including miR-34b, miR-326, miR-432, miR-548c-3p, miR-570 and miR-603, that were drastically and consistently down-regulated in GH adenomas and demonstrated that these miRNAs target genes with critical roles in pituitary tumourigenesis, such as high-mobility group A1 (*HMG A1*), *HMG A2* and *E2F1*. Butz *et al.* (98) demonstrated that *Wee1*, a nuclear protein that delays mitosis and was recently recognised as a tumour suppressor gene, is down-regulated by a set of three miRNAs that are up-regulated in NFPAs (miR-128a, miR-155 and miR-516a-3p), thus suggesting that the regulation of *Wee1* kinase by miRs may be linked to pituitary tumourigenesis.

However, only one study (106) comparing pituitary adenoma to pituitary carcinoma has correlated miRNA deregulation with tumour behaviour. Another study (102) analysed the miRNA expression in GH tumour and identified nine miRNA that were differentially expressed between micro vs macroadenoma, and seven miRNA that were differentially expressed between tumours that were responsive and those not to lanreotide treatment.

DNA methylation

The impact of methylation on pituitary tumourigenesis has been recently evaluated by quantitative genome-wide analysis of the DNA methylome in 32 sporadic pituitary adenomas (seven GH, six ACTH, six PRL and 13 NFPA) compared with normal pituitary (109). Differential methylation across and between adenoma subtypes identified 12 genes, but an inverse relationship between methylation and transcript expression was observed for only three (*EML2*, *RHOD* and *HOXB1*). Among the genes specifically methylated in NFPAs, hypermethylation of two (*KIAA1822* (*HHIPL1*) and *TFAP2E*) had been validated in an independent cohort. The functional characterisation of the identified genes will also provide insights into tumour aetiology and more studies are needed to evaluate the impact of methylation on pituitary tumour behaviour.

Genetic markers of aggressiveness

Pituitary tumours are mostly sporadic, but some familial cases have been identified, leading to the characterisation of genetic forms of pituitary adenoma. The search for

MEN1 or *AIP* mutation is necessary in patients with a familial history (110, 111, 112) or in young patients with a large macroadenoma (111, 113, 114), or in case of unusual plurihormonality or double adenomas (114). Other than these indications, clinicians should be aware of possible sporadic mutation in case of aggressive tumours, following recent evidence of tumours associated with *MEN1* or *AIP* mutation being more commonly resistant to conventional treatment (115, 116). *AIP* immunostaining may also be predictive of the response to somatostatin analogue in GH-secreting tumours (117, 118).

Therapeutic applications

Recent clinical trials have demonstrated the successful application of a new chemotherapeutic agent, temozolomide, to treat the rare pituitary carcinomas, suggesting that this drug may be potentially useful as an 'aggressive strategy' to treat the group of 'aggressive' tumours with a high risk of recurrence or resistance. Indeed, this alkylating agent, frequently used to treat glioblastoma or some endocrine tumours (119, 120), may be efficient to control tumour progression and metastasis in about 50–60% of cases (reviews (5, 6)). There is some evidence of the treatment outcome depending on the expression of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme that potentially interferes with drug efficacy (11, 121). However, a recent review did not support such a predictive value of MGMT expression, suggesting that it should not be considered a reason to deny patients the potential benefit of temozolomide treatment (5, 122, 123). Encouraging results from Hirohata *et al.* (123) suggested that immunopositivity of DNA mismatch repair protein (MSH6) was positively correlated with response of 14 atypical pituitary adenoma or pituitary carcinomas to temozolomide. None of p53, Ki-67 or MGMT expression showed any significant correlation with the efficacy of temozolomide.

Despite these encouraging results with temozolomide treatment showing long-term control for some patients (12, 122, 124, 125, 126), temozolomide is not effective for all pituitary carcinomas or aggressive adenomas, and some tumours develop secondary resistance during follow-up (5, 6, 12). The development of new therapeutic options is therefore necessary (10). Some case reports suggest the need to associate temozolomide with other chemotherapeutic agents (127, 128) or with the new somatostatin analogue pasireotide (129), while other preclinical and clinical studies suggest that new targeted therapies may be useful for controlling pituitary tumour growth. Indeed,

Raf/MEK/ERK and PI3K/Akt/mTOR pathways are up-regulated in their initial cascade in pituitary tumours (130), and the anti-proliferative effect of mTOR inhibitor on different pituitary cell lines or primary cultures has been demonstrated *in vitro* (131, 132, 133), though not in humans (10). Some evidence does exist supporting the role of the EGFR pathway (134, 135), and the potential of tyrosine kinase inhibitors (136, 137) as good candidates for such targeted therapies. However, so far, the outcome of only one patient, in whom tumour growth has been controlled by anti-VEGF, has been published (138). Despite this condition being rare, new therapeutic options are needed for these patients.

Recent advances in pituitary tumour classification now allow the early identification of pituitary tumours with a high risk of recurrence and resistance to conventional treatment strategies, associated with a malignant potential. In such cases (grade 2b), after discussion implicating a designated team of neurosurgeons, endocrinologist, pathologist and oncologist, an optimised therapeutic strategy should be proposed taking into account new therapeutic options in addition to conventional therapies associating surgery and radiotherapy (139).

Conclusion

The evidence against pituitary tumours being considered only as benign tumours inducing hormonal disease is mounting. Besides, the rare carcinomas and the frequent non-invasive, non-proliferative benign pituitary tumours, a group of tumours representing almost 15% of all pituitary tumours, should be individualised. The validity of the term 'atypical tumours' proposed by the 2004 WHO pituitary tumour classification is now debatable. The term aggressive tumours referred to a clinical definition, which was mostly based on the follow-up and could not therefore be used for predicting tumour behaviour and therapeutic management. Consequently, we propose naming such grade 2b tumours with a high risk of recurrence as 'tumour suspected of malignancy'. Indeed, clinical, pathological and molecular evidence suggests that some of these tumours may develop metastasis during follow-up and should be treated with a more intensive and personalised therapeutic strategy. Recent genomic studies identifying the new molecular markers associated with tumourigenesis may help to identify new targeted therapies. Among these markers, the identification of specific genomic alterations, easily studied on fixed tissue, seems promising to allow a better classification of these patients with high risk of recurrence.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

This work was supported by grants from the Ministère de la Santé (Programme Hospitalier de Recherche Clinique National no. 27-43, HYPO-PRONOS) and research contracts with the Institut National de la Santé et de la Recherche Médicale and the Ligue Contre le Cancer Rhône-Alpes.

Acknowledgements

The authors thank Emily Witty from Angloscribe for helping with the English translation, P Gerardi for typing the manuscript, P Chevallier, A Reynaud and M P Guigard for technical assistance and all the members of HYPOPRONOS: R Assaker, C Auger, A Barlier, M Bernier, F Bonnet, J F Bonneville, F Borson-Chazot, G Brassier, T Brue, S Caulet-Maugendre, O Chabre, F Chapuis, P Chanson, A Cornelius, C Cortet-Rudelli, J F Cottier, E Dantony, B Delemer, E Delgrange, L Di Tommaso, H Dufour, S Eimer, D Figarella-Branger, P François, S Gaillard, F Galland, J J Girard, M Jan, J Lachuer, V Lapras, H Loiseau, C A Maurage, F Mougél, J G Passagia, M Patey, A Penfornis, J Y Poirier, G Perrin, P Roy, N Sturm, A Tabarin, L Villeneuve, G Viennet and A Wierinckx.

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Received 16 December 2013

Revised version received 12 January 2014

Accepted 15 January 2014