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The 2004 World Health Organization classification of pituitary tumors: What is new?

Received: 17 May 2005 / Revised: 17 May 2005 / Accepted: 18 May 2005 / Published online: 23 November 2005
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Abstract The classification of pituitary tumors is a dynamic area that changes with advances in cell biology that provide a deeper insight and clearer understanding of cell lineages and pathogenetic mechanisms. The 2004 edition of the World Health Organization (WHO) text “Histological typing of endocrine tumors” reflects the progress that has been achieved since the previous edition of 2000. Here we review the new information and identify areas of concern for the next effort at classification of pituitary tumors.

Keywords Pituitary · Classification · Molecular · Differentiation · Immunocytochemistry

Introduction

Pituitary neoplasms are relatively common tumors [26] that exhibit a wide range of hormonal activities and proliferative behaviors. Classification of these tumors is a dynamic area requiring continuous critical review due to advances in molecular genetic techniques and new immunohistochemical stains that provide a deeper insight and clearer understanding of cell lineages and pathogenetic mechanisms in adenohypophysial tumors. The 2004 edition of the World Health Organization (WHO) text “Histological typing of endocrine tumors” [19] reflects the progress that has been achieved since the previous edition of 2000. Data on the biological factors implicated in pituitary tumorigenesis have been extensively explored and putative oncogenes and tumor

suppressor genes have been identified. Some pituitary adenoma subtypes have been reclassified. More pathological markers were postulated to predicate the biological behavior of the pituitary adenomas. In the past, the concept of plurihormonality was poorly understood. The identification of transcription factors that regulate cell differentiation and hormone production allows a framework to classify pituitary tumors by their cytodifferentiation and to explain patterns of plurihormonal gene expression.

What is new in tumor classification?

The last decade or more has seen a large body of literature emerge concerning the transcription factors that regulate cell differentiation and hormonal activity of adenohypophysial cells [3, 5, 7]. Most of this work has examined embryological development, and the application of gene deletion technology has allowed mouse models to prove the role of specific proteins in organogenesis and cytodifferentiation. The data provide new information about tumors that arise from adenohypophysial cells as well as other diseases such as congenital abnormalities [4].

It has become clear that there are three main pathways of cell differentiation in the anterior pituitary. Adrenocorticotrophic hormone (ACTH)-producing corticotrophs are determined by the expression of Tpit that binds with corticotropin upstream transcription-binding element (CUTE) proteins including neuroD1/beta 2. Bihormonal gonadotrophs require expression of steroidogenic factor (SF)-1 (Fig. 1a), GATA-2 and members of the Lhx gene family, particularly Lhx4. The complex family of Pit-1 expressing cells can mature into somatotrophs (Fig. 1b), mammosomatotrophs, lactotrophs or thyrotrophs with the additional expression of estrogen receptor (ER) alpha, which enhances prolactin (PRL) secretion, or thyrotroph embryonic factor (TEF), which stimulates thyroid-stimulating hormone (TSH)-beta production. The recognition of these molecular

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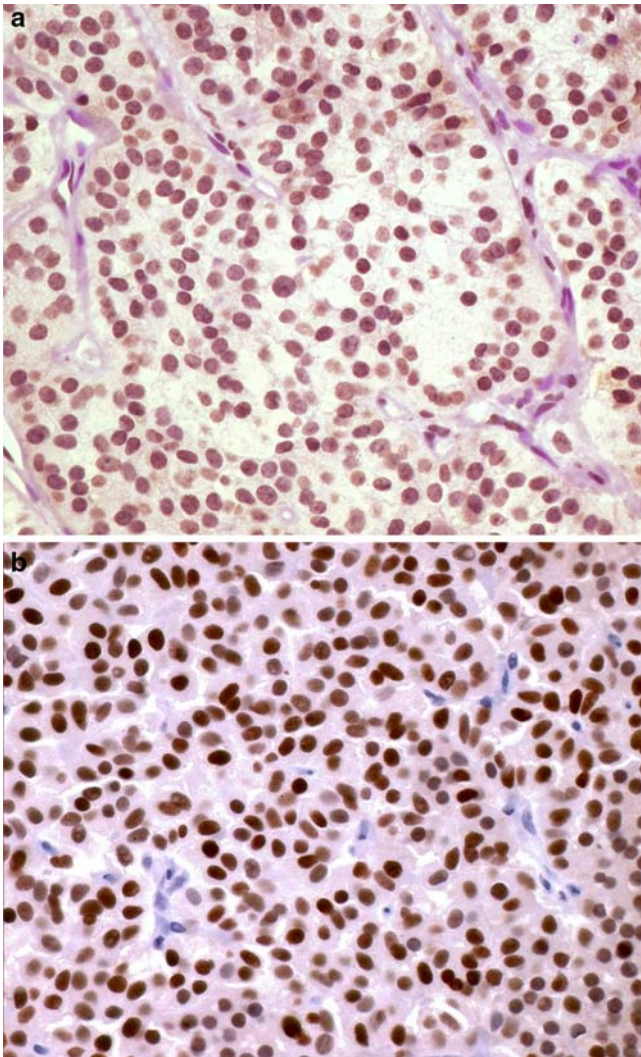


Fig. 1 Transcription factor determination of pituitary adenoma cytogenesis. (a) This clinically nonfunctioning pituitary adenoma exhibits nuclear reactivity for SF-1. This stain identifies this as a tumor of gonadotroph differentiation. (b) The intense nuclear Pit-1 reactivity of this tumor narrows the diagnosis of this lesion to a member of the GH/PRL/TSH family of adenomas

determinants of adeno-hypophysial cytodifferentiation has clarified the cytogenesis of a number of unusual tumors, and has explained patterns of plurihormonality which have been recognized in pituitary adenomas, thereby providing a framework for classification of these tumors (Table 1).

One of the areas in which this information has helped is in the area of hormonally inactive adenomas. These have been classified as null cell adenomas and oncocytomas in the past, but indeed, at the time of their description, Kovacs et al. [40] predicted that the terms would become obsolete as new markers emerged to classify them further. The expression of SF-1 by the majority of these adenomas (Fig. 1a) has allowed them to be classified as members of the gonadotroph adenoma family [10]. This is not a surprise, since it has been

known for some 20 years that null cell adenomas and oncocytomas release gonadotropins in vitro [8, 9, 63], but this hormonal activity is often not detected by conventional immunohistochemistry. The data provide evidence for the application of new antisera that allow cytogenetic classification in the absence of hormone positivity.

In rare instances, silent adenomas may express Pit-1 or Tpit, and these features alone will predict a different biology. The finding of one of these transcription factors in a clinically silent and hormone immunonegative adenoma proves pituitary origin and points to possible therapeutic applications. Future studies will allow us to prove whether hormone-negative Tpit-positive silent adenomas are as aggressive as the ACTH-reactive silent corticotroph adenomas [32, 39], and whether somatostatin analogues could prove useful in the management of Pit-1-positive silent adenomas.

A controversial entity has been the tumor classified as “female gonadotroph adenoma”. This lesion was described on the basis of an unusual pattern of the Golgi complex, the so-called “honeycomb” Golgi, that is seen mainly by electron microscopy [31]. The fact that a few of these lesions contained immunoreactive gonadotropin subunits led to the classification scheme; however, recent studies have proven that this alteration of the Golgi complex can also occur in cells of corticotroph lineage that express Tpit [51, 52].

The group of plurihormonal adenomas has also undergone refinement of classification. This is based on two major advances. Firstly, the understanding of pathways of cytodifferentiation has firmly established the relationships between cells that produce different hormones. For example, tumors that produce growth hormone (GH) and PRL have long been recognized to derive from normal mammosomatotrophs, but the relationship of TSH to this family of lesions, as occurs occasionally by immunohistochemistry and more rarely with the clinical manifestation of hyperthyroidism in acromegaly, was an enigma. Such tumors were classified as unusual plurihormonal adenomas. Today, we recognize these to be manifestations of Pit-1 expression. Secondly, the recognition in the early 1990s that polyclonal antisera often were contaminated with antibodies to alpha-subunit of the glycoprotein hormones paved the way for a better technical approach to immunoprofiling of pituitary adenomas [41]. Since alpha-subunit is normally expressed in somatotrophs, thyrotrophs and gonadotrophs, the presence of this protein often led to the identification of gonadotropins in tumors of the Pit-1 family. The use of new monoclonal antisera prevents this artifact and allows a more accurate classification of tumors.

There remain rare adenomas that truly exhibit hormone immunoreactivities that cross the lines of cytodifferentiation. An example is the so-called “silent subtype 3 adenoma”. This rare lesion occurs in both genders, tends to present as a macroadenoma and has an aggressive clinical behavior [32, 33]. The clinical

Table 1 Classification of pituitary adenomas by cytodifferentiation (*ER* estrogen receptor, *GH* growth hormone, *PRL* prolactin, *TEF* thyrotroph embryonic factor, *TSH* thyroid-stimulating hormone, *ACTH* adrenocorticotropic hormone, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *SF* steroidogenic factor)

Tumor type	Transcription factors	Hormones, others
The Pit-1 family		
Somatotroph adenoma		
Densely granulated somatotroph adenoma	Pit-1	GH, α -subunit
Sparsely granulated somatotroph adenoma	Pit-1	GH, keratin whorls (fibrous bodies)
Mammotroph/mixed adenoma	Pit-1, ER	GH, PRL, α -subunit
Lactotroph adenoma		
Sparsely granulated lactotroph adenoma	Pit-1, ER, ?GH-repressor	PRL, Golgi pattern
Densely granulated lactotroph adenoma	Pit-1, ER, ?GH-repressor	PRL diffuse cytoplasmic
Acidophil stem cell adenoma	Pit-1, ER	PRL, (GH), keratin whorls (fibrous bodies)
Thyrotroph adenoma	Pit-1, TEF, GATA-2	β -TSH, α -subunit
Plurihormonal adenoma	Pit-1, ER, TEF, GATA-2	GH, PRL, β -TSH, α -subunit
ACTH family		
Corticotroph adenoma	Tpit	ACTH, keratins
Gonadotropin family		
Gonadotroph adenoma	SF-1, ER, GATA-2	β -FSH, β -LH, α -subunit
Unclassified adenoma		
Hormone-negative/ null cell adenoma	None	None
Unusual plurihormonal adenoma	?multiple	Multiple

presentation is highly variable, depending on the hormone produced by the adenoma. Morphologically these tumors tend to be chromophobic and are usually PAS negative; they are characteristically composed of spindle cells with a fibrous stroma. Nuclear pleomorphism and mitosis can be present. Immunohistochemically they can contain almost any combination of pituitary hormones; however, the most common pattern is GH, PRL, and TSH, with occasional tumors expressing gonadotropins. Further studies of transcription factors and the application of stringent specificity in immunophenotyping will provide clarification of the cytogenesis of these lesions.

What are the currently accepted prognostic and predictive factors?

New data have begun to shed light on the prognosis of pituitary adenomas. The proliferative activity of pituitary tumors has been extensively investigated [2, 38, 59]. Studies of proliferating cell nuclear antigen (PCNA), Ki-67/MIB-1, and anti-apoptotic Bcl-2 have unfortunately demonstrated no consistent correlation with tumor invasiveness or recurrence [2]. DNA topoisomerase II alpha (Topo II alpha), another marker of cell proliferation, was investigated in pituitary adenomas and carcinomas in relation to their biological behavior; although invasive pituitary adenomas and carcinomas exhibit a high Topo II alpha index, this indicator has no significant advantage over MIB-1 as a prognostic marker [61]. Cyclooxygenase-2 (COX-2) expression correlates with patient age, but not with tumor size or invasiveness [62]. Detection of telomerase expression may predict recurrence in pituitary adenomas [64]. Galectin-3, a beta-galactoside-binding protein implicated in cellular differentiation and

proliferation as well as angiogenesis, tumor progression and metastasis, may play a role in pituitary tumor progression [50].

Unfortunately, none of these is truly a marker of biological behavior. The best predictive markers remain those that subclassify adenomas accurately based on hormone content and cell structure. For example, among acromegalics who fail surgical resection, response to long-acting somatostatin analogues is best predicted by the subtype of somatotroph adenoma as densely or sparsely granulated [15, 21]. This finding renders the value of a Cam 5.2 keratin stain more important than almost any other immunostain in this setting. A silent corticotroph adenoma will recur more often and more aggressively than a silent gonadotroph adenoma. In addition, a silent subtype 3 adenoma will almost certainly behave invasively, infiltrating the base of the skull, while a silent adenoma of the gonadotroph lineage will usually grow by expansion upwards.

What are the recent advances in the pathobiology and molecular alterations underlying pituitary adenoma formation?

Our understanding of pituitary tumorigenesis is largely derived from experimental studies in animal models and from molecular analyses of human pituitary tumors. Alterations of proto-oncogenes and tumor suppressor genes have been implicated in the pathogenesis of these tumors [6] (Table 2).

Activating mutations of the Gs-alpha subunit gene on chromosome 20 [42, 57, 58] have been demonstrated mainly in a subset of GH-producing adenomas. Overexpression of the epidermal growth factor receptor (EGF-R) has also been implicated in the more aggressive behavior of recurrent somatotroph adenomas [43].

Table 2 Putative oncogenes and tumor suppressor genes implicated in pituitary tumorigenesis (*EGF* epidermal growth factor, *EGF-R* EGF receptor, *FGF* fibroblast growth factor, *TGF* transforming growth factor, *PTTG* pituitary tumor transforming gene, *ptd* pituitary tumor-derived)

Oncogenes	Tumor suppressor genes
Gsz	GADD45
ras	Menin
CREB	p16/CDKN2A
Cyclin D1	p18
EGF/TGF-alpha/EGF-R	p27/KIP1
FGF-2, FGF-4	Rb
PTTG	Tp53
ptd-FGFR4	

Another member of the EGF family, TGF-alpha, is overexpressed in some pituitary adenomas [23] and TGF-alpha overexpression in lactotrophs has been shown to result in prolactinoma formation in transgenic mice [45].

Fibroblast growth factor (FGF)-2 (basicFGF, bFGF) and FGF-4 have been implicated in prolactinoma development [16, 53, 54], but FGF-2 is also produced by other adenomas, and elevated circulating levels are associated with aggressive pituitary tumors [20, 22]. Another novel mechanism of FGF function in pituitary adenomas involves an antisense gene that regulates cell proliferation and hormone secretion [12]. Altered FGF receptor expression has been found in pituitary adenomas [1], and the fourth member of this family, FGFR4, undergoes alternative transcription initiation in pituitary adenomas, giving rise to an oncogenic protein [24] in pituitary adenomas of various subtypes. Expression of this pituitary tumor-derived (ptd)-FGFR4 protein is more frequent in macroadenomas than in microadenomas and correlates with the Ki-67 labeling index [49]. Recent data suggest that ptd-FGFR4 alters cell adhesion by a mechanism that explains the loss of reticulin, which is the hallmark of pituitary adenomas [27].

Other putative oncogenes in pituitary adenomas include cyclin D1 overexpression [30, 36] and upregulation of pituitary tumor transforming gene (PTTG), a member of the securin family of proteins involved in chromosome separation during cell division [47, 68]. PTTG overexpression correlates with FGF-2 overexpression [16]. Mutations of the ras genes have been implicated in the development of rare pituitary carcinomas [48].

The loss of tumor suppressor gene expression in the pituitary is a critical event in endocrine tissues and specifically pituitary, as shown by animal studies. *p27^{kip1}*-null mice develop multiorgan neoplasia, including pituitary tumors [28, 37, 46]. Mice lacking both *p18^{ink4}* and *p27^{kip1}* succumb to lethal pituitary adenomas by 3 months of age [29]. The expression of p27 is reduced in pituitary adenomas, mainly in ACTH-producing tumors [14, 18, 35, 44]. Hypermethylation of p16 is detected in adenomas of gonadotroph lineage [55]. In contrast to these models, another mouse model of

pituitary tumorigenesis, deficiency of the retinoblastoma (Rb) gene, does not seem to have human application. While the mice develop pituitary corticotroph adenomas arising in the intermediate lobe [34], human tumors have no mutation or loss of heterozygosity (LOH) at the Rb locus [56, 66]. This may be attributed to unique features of intermediate lobe corticotrophs that do not apply in humans.

Since pituitary adenomas form an important part of the syndrome of multiple endocrine neoplasia type 1 (MEN-1), the menin gene has been thought to be a critical tumor suppressor for pituitary. Although familial pituitary adenomas associated with MEN-1 have mutation and LOH of the menin locus at 11q13, these events and even down-regulation of menin are rare or absent in the more common sporadic adenomas [11, 67].

The p53 gene does not seem to be of pathogenetic significance in pituitary tumors. Although p53 expression has been detected in adenomas of all types, and may be more common in recurrent neoplasms [17, 60], there is no evidence of mutation or LOH and the significance of the immunoreactivity remains to be established.

GADD45 gamma is a member of a growth arrest and DNA damage-inducible gene family that functions in the negative regulation of cell growth. The mRNA expression of the GADD45 gamma gene is significantly reduced in clinically nonfunctioning pituitary adenomas using cDNA-representational difference analysis [65] and this too has been shown to be due to promoter methylation [13].

The vast majority of the genetic alterations in pituitary adenomas appear to be due to promoter methylation or acetylation with silencing of tumor suppressors. A role for the chromatin-remodeling protein Ikaros has been identified in the genesis of the oncogenic ptd-FGFR4 [25]. This takes the analysis of the molecular basis of pituitary tumorigenesis to the level of epigenetic regulation.

Conclusions

The 2004 edition of the WHO histological typing of endocrine tumors [19] has illustrated tremendous progress in our understanding of pituitary tumors. However, there is much that remains to be learned.

There are major weaknesses that remain in our approach to these lesions. The WHO book includes only three accepted types of primary adenohypophysial lesion: typical pituitary adenoma, atypical pituitary adenoma and pituitary carcinoma. Buried in the text of the introduction is a short paragraph indicating that tumors with invasive growth, elevated mitotic index, Ki-67 labeling index >3% and extensive nuclear reactivity for p53 are considered "atypical". Despite this, and reflecting the consensus of the contributors, the bulk of the text provides the detailed classification of ade-

nomas as shown in Table 1. The observant reader will note a lack of ICD codes for these distinct and clinically relevant lesions; ICD codes are only provided for typical pituitary adenoma (8272/0), atypical pituitary adenoma (8272/1) and pituitary carcinoma (8272/3). As indicated above, the most important clinical and prognostic features of pituitary adenomas remain the hormonal profile and subtype classification. The lack of accurate codes to reflect this classification will result in a continuing inability to collect statistics and represents a failure on the part of the pathology community to play an important role in clinical epidemiology.

In contrast to almost all other tumors classified in the various texts of this series, there is a conspicuous lack of malignant lesions among the primary pituitary tumors. The text does include a comprehensive list of sarcomas, lymphomas and metastatic carcinomas that do occur in the sella turcica. However, the diagnosis of primary pituitary carcinoma remains based solely on the identification of distant metastasis. While this facilitates the diagnostic approach to these tumors, it seems inappropriate. It is ironic that basal cell carcinoma of skin is classified as a cancer, while these lesions, that can cause significant mortality due to hormone hypersecretion, tumors that too often invade the brain, cause blindness and nerve palsies, require radiotherapy, and ultimately cause death in some patients, are not within the jurisdiction of cancer agencies. The spectrum of biological behavior that they display and the linkages or lack thereof between differentiated activity and cell proliferation makes pituitary adenomas an ideal model for the study of mechanisms of tumorigenesis. It behooves the endocrine community to advocate for a reexamination of the criteria of malignancy as they apply to pituitary neoplasia.

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