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ARTICLE

Epidemiology and biomarker profile of pituitary adenohypophysial tumors

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Abstract

Recent studies have reported the prevalence of pituitary tumors to be ~1/1000 population. Many are prolactin-producing tumors that are managed medically, however, the epidemiology of surgically resected pituitary adenohypophysial neuroendocrine tumors has not been reported in a large series with detailed characterization. We reviewed 1055 adenohypophysial tumors from 1169 transsphenoidal resections from the pathology files of University Health Network, Toronto, 2001-2016. Tumors were characterized by immunohistochemical localization of transcription factors (Pit-1, ERα, SF-1, Tpit), hormones (adrenocorticotropin, growth hormone, prolactin, β-thyrotropin, β-folliculotropin, β-luteotropin, α-subunit), and other biomarkers (keratins, Ki67, p27, FGFR4). Electron microscopy was used only for unusual lesions. In this cohort, 51.3% of patients were female; the average age was 51 years. Gonadotroph tumors represented 42.5%. Pit-1-lineage-tumors represented 29.9%; these were subclassified as growth-hormone-predominant (somatotroph/mammosomatotroph/mixed; 53%), prolactin-predominant (lactotroph/acidophil-stem-cell; 28%), thyrotrophs (2%), plurihormonal (14%), and not-otherwise-specified (3%). Corticotroph tumors represented 17.1%. Only 4.5% were null cell tumors and 0.5% were unusual plurihormonal tumors. In 5.5% the tumor was not characterized for technical reasons (sample size, fixation, necrosis or other artifact). All corticotroph and plurihormonal tumors were positive for keratins; others tumors showed variable negativity with highest rates in gonadotroph (37.1%) and null cell tumors (28.2%). Tumors with a Ki67 ≥ 3% comprised 60% of this cohort. Global loss of p27 was most frequent in corticotroph neoplasms, specifically those associated with elevated glucocorticoid levels. Corticotroph and lactotroph tumors were more common among females; gonadotroph tumors were more common among males. Younger patients had mainly corticotroph and Pit-1-lineage neoplasms, whereas older patients harbored mainly gonadotroph tumors. This represents one of the largest surgical series of morphologically characterized pituitary tumors reported to date and the first to include the routine use of transcription factors for tumor classification. The data provide the basis for clinicopathologic correlations that are helpful for prognostic and predictive patient management.

Introduction

Pituitary tumors have been considered rare in the past but are increasingly recognized as a common disorder. Epidemiologic data prior to 1969 indicated an annual incidence of 1.85 per 100,000 population [1]. Reports of clinical series from some experts indicated that they constituted 10 to 25% of intracranial neoplasms [2] but these studies may have reflected individual practice bias. Reports from radiology and from pathology autopsies reported common incidental findings of pituitary lesions and in 2004, a meta-analysis of these data revealed that they occur in almost 17% of the population [3]. These "incidentalomas" prompted studies of the prevalence of pituitary tumors. At least 5 studies have reported a prevalence of approximately 1/1000 population [4–8].

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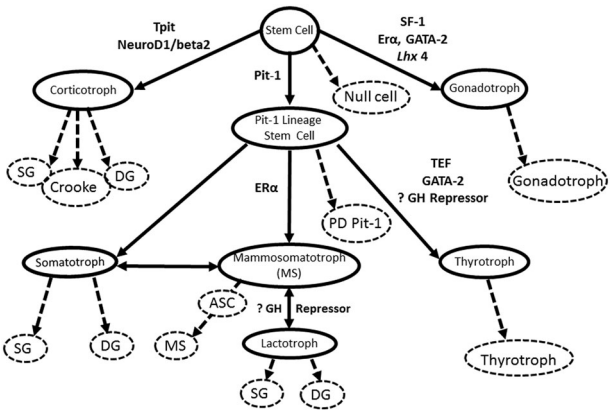

Fig. 1 Pituitary adenohypophysial neuroendocrine tumor cytogenesis. The development of pituitary cells follows three lineages that are dependent on expression of transcription factors that are required for hormone gene expression. Corticotrophs require Tpit and NeuroD1/beta-2 for their differentiation; they give rise to tumors that are generally classified into three types, densely granulated (DG) basophilic corticotroph tumors that resemble normal corticotroph, sparsely granulated (SG) tumors that are chromophobic and Crooke cell tumors that resemble the suppressed nontumorous corticotrophs in patients with glucocorticoid excess. Gonadotrophs require SF-1, ER α, GATA-2, and *Lhx* 4 to develop; they develop tumors that are relatively homogeneous morphologically. The Pit-1 family is the most complex with 4 mature cell types: somatotrophs, mammosomatotrophs (MS), lactotrophs and thyrotrophs, each of which gives rise to tumors, again with variants that are DG or SG in some cases. Poorly differentiated tumors of Pit-1 lineage (PD Pit-1) are likely to arise from a Pit-1 stem cell, acidophil stem cell (ASC) tumors are thought to be primitive mammosomatotroph tumors, and null cell tumors likely arise from an adenohypophysial stem cell

Pituitary tumors of adenohypophysial cell differentiation, recently renamed pituitary neuroendocrine tumors [9], exhibit a wide range of clinical manifestations and behaviors. The prevalence data indicate that approximately one third are prolactin-producing tumors that are managed medically. However, the remainder are surgically resected and subjected to surgical pathology classification. With increasingly sophisticated tools to analyze these specimens, pathologists have achieved a classification that has prognostic and predictive value [10].

We report a retrospective analysis of a series of surgically resected pituitary adenohypophysial tumors collected over a 15-year period at a single center to determine the epidemiology and biomarker profile applied with the most current tools for the classification of tumors of adenohypophysial differentiation based on cytogenesis (Fig. 1).

Materials and methods

We obtained ethics approval from the Research Ethics Board of the University Health Network to search the files of the laboratory information system of the Department of Pathology for all specimens identified as "pituitary" between 2001 and mid-July 2016. All cases of craniopharyngioma, sellar cysts, inflammatory lesions, pituicytomas, metastatic carcinomas and tumors of other cell types were excluded, leaving 1055 pituitary adenohypophysial neuroendocrine tumors from 1169 transsphenoidal resections.

Routine clinical practice has been to characterize all tumors using a panel of stains. Histochemical stains were the periodic acid Schiff (PAS) and Gordon-Sweet silver stain for reticulin. Immunohistochemical localization (Supplementary Table 1) was performed for transcription factors including Pituitary transcription factor-1 (Pit-1), Steroidogenic Factor-1 (SF-1) and Estrogen Receptor alpha (ER). A panel of hormone immunostains was used to localize adrenocorticotropin (ACTH), growth hormone (GH), prolactin (PRL), alpha-subunit of glycoprotein hormones (αSU), beta-thyrotropin (βTSH), beta-folliculotropin (β-follicle stimulating hormone; βFSH), and beta-luteotropin (β-luteinizing hormone; βLH). Because of variable glycosylation of the α-subunit, two stains were routinely performed, one against the pituitary α-subunit itself and one against α-human chorionic gonadotropin (αhCG) that is highly homologous. Select cases were also tested for expression of the T box transcription factor that is the product of *TBX19* known as Tpit. Keratins were routinely localized using the CAM5.2 antibody that identifies keratins 8 and 18, but in some cases, including those that were negative with CAM 5.2, additional stains were performed using the AE1/AE3 cocktail that identifies pankeratins. Tumors were also routinely stained for p27 and FGFR4, and the MIB-1 antibody was used to localize Ki-67. The Ki67 labeling index was calculated as the percentage of positive cells over total nuclei. FGFR4 and p27 were scored as negative (0), focal or patchy (1) and diffusely positive (2); calculated averages were obtained. Electron microscopy was used only for unusual lesions.

Pathology reports included data collected from the clinical charts or referring physicians and surgeons when it was available as well as the morphologic, histochemical and immunohistochemical features; since 2008, these data were collected in a synoptic searchable database [11]. The data were collected in an Excel spreadsheet and analyzed. Diagnostic data analysis was facilitated through a process of data coding into 10 major diagnostic types including Pit-1 lineage growth hormone--predominant (somatotroph/mammosomatotroph/mixed somatotroph-lactotroph) tumor, Pit-1 lineage prolactin-predominant (lactotroph/acidophil stem cell) tumor, Pit-1 lineage thyrotroph tumor, poorly differentiated Pit-1 lineage tumor (formerly known as silent subtype 3 adenoma), Pit-1 lineage tumor not-otherwise-specified, corticotroph tumor, gonadotroph tumor, null cell tumor, plurimorphous plurihormonal tumor, and pituitary neuroendocrine tumor not-otherwise-specified. The somatotroph, lactotroph, and corticotroph tumors were further subtyped based on their characteristics. Summary statistics frequencies were found for all the nominal and ordinal

variables studied here and frequency tables and graphs produced for purposes of quantification and crosschecking. The comparison of p27 scores within the group of corticotroph tumors and comparison of FGFR4 scores among all tumor groups were assessed using the t-test. The comparison of tumor size and FGFR4 scores was assessed using the Pearson correlation test. The sensitivity, specificity, positive and negative predictive value of SF-1, ER, α-subunit, α-human chorionic gonadotropin, β-follicle stimulating hormone and β-luteinizing hormone were assessed for the diagnosis of gonadotroph lineage.

Results

In this cohort, 51.3% of patients were female; the average age was 51 years. The youngest patient was 8 years old and the oldest was 88.

In 59 cases (around 5.5% of the entire cohort), the tumor could not be accurately characterized due to technical limitations including small tissue size, inadequate fixation, complete necrosis or other artifacts. In 13 patients, there were multiple pituitary adenohypophysial tumors; 12 were double and one patient had three distinct synchronous tumors. In all we analyzed 1055 tumors from 1169 surgical procedures. The larger number of surgical procedures reflects multiple surgical procedures for 69 persistent/recurrent tumors and 30 procedures that did not yield tumor tissue.

Tumor classification

Tumor classification by cytogenesis is illustrated in Fig. 2.

Epidemiology by tumor type

The distribution of tumor types by age is illustrated in Fig. 3; gonadotroph and null cell tumors that present as clinically nonfunctioning masses are rare in young patients and increase with age, whereas prolactin-producing tumors are common in youth and decrease with age. Interestingly, growth hormone-secreting tumors predominantly occur between the ages of 20 and 50 years, while corticotroph tumors occur at any age. Gender incidences are shown in Fig. 4; women predominate at early ages and most markedly among corticotroph and somatotroph tumors.

Features of individual tumor types

Gonadotroph tumors

Gonadotroph tumors represented 42.5% of the cohort. Among the 448 tumors, 63% were in males; the mean age was 58 years. These tumors were typically classified based

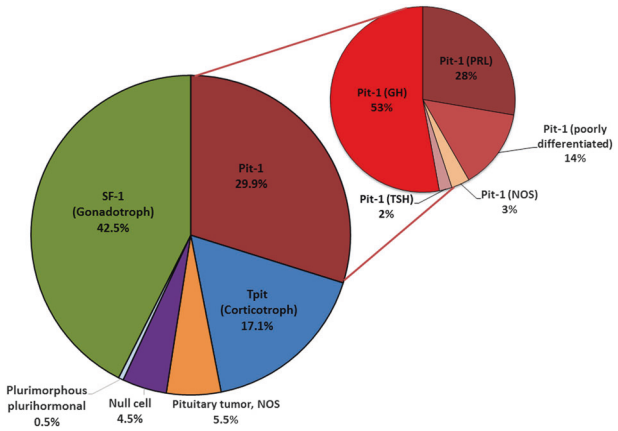

Fig. 2 Cytogenesis of 1055 pituitary adenohypophysial neuroendocrine tumors. The majority of surgically resected tumors are of gonadotroph lineage; Pit-1 lineage tumors comprise the second largest group and their breakdown is shown in the small pie chart at right. Corticotroph tumors are the third largest group with only small percentages of null cell and unusual plurihormonal tumors. The group of unclassified tumors (not otherwise specified, NOS) represent tumors that could not be classified for technical reasons, usually insufficient, crushed or poorly fixed tissue. (*GH* growth hormone, *PRL* prolactin, *TSH* thyrotropin, *ACTH* adrenocorticotropin)

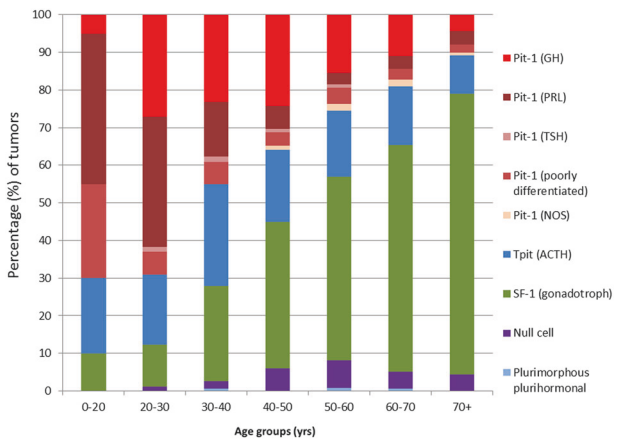

Fig. 3 Age distribution of pituitary adenohypophysial neuroendocrine tumor subtypes. There are clear differences in the distribution of tumors that arise at different ages. Note the increase in gonadotroph tumors with age and the predominance of Pit-1 lineage tumors in younger patients. Tumors lacking a subtype diagnosis were excluded from this figure. (*GH* growth hormone, *PRL* prolactin, *TSH* thyrotropin, *NOS* not otherwise specified, *ACTH* adrenocorticotropin)

on expression of gonadotroph cell-lineage transcription factors (SF-1, ER) and/or gonadotropin immunoreactivity. Staining for SF-1 was identified in 96% of these tumors; ER was positive in 82%. Fourteen tumors were negative for both SF-1 and ER; eleven of those showed reactivity for gonadotropins and/or α-subunit, and the remaining three tumors with no reactivity for hormones revealed morphological and ultrastructural characteristics of gonadotroph tumors. Hormone reactivity was less common as follows:

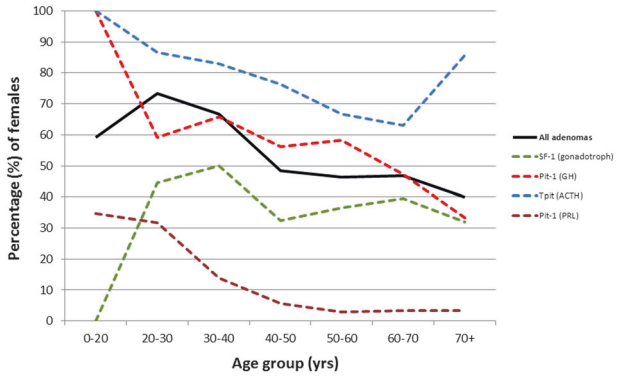

Fig. 4 Pituitary adenohypophysial neuroendocrine tumor type by gender and age. Tumor subtypes with fewer than 2 specimens per age/gender group were excluded from this figure. (*GH* growth hormone, *PRL* prolactin, *TSH* thyrotropin, *ACTH* adrenocorticotropin)

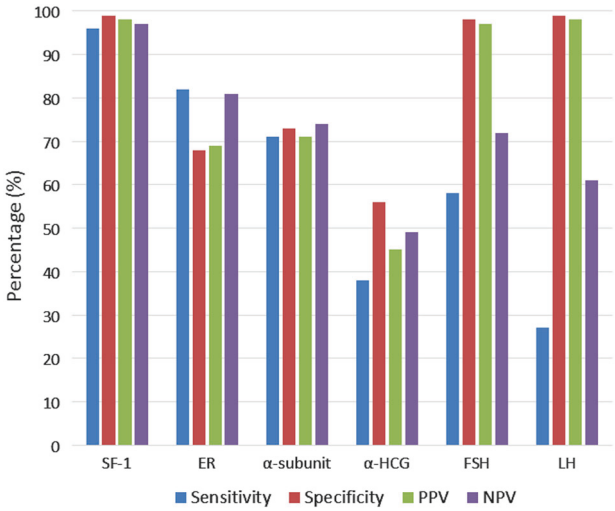

Fig. 5 Biomarkers of gonadotroph tumors. Gonadotroph tumors may be negative for hormones on immunohistochemical staining. This graph shows the sensitivity, specificity, positive and negative predictive values (PPV, NPV) of the various biomarkers of these tumors. (*SF-1* steroidogenic factor-1, *ER* estrogen receptor *hCG* human chorionic gonadotropin, *FSH* follicle stimulating hormone, *LH* luteinizing hormone)

α-subunit in 71%, β-follicle stimulating hormone in 58.7%, α-human chorionic gonadotropin in 38% and β-luteinizing hormone in 27.4%. Because these cells are capable of expressing both β-follicle stimulating hormone and β-luteinizing hormone, we calculated the profile of both hormones; β-follicle stimulating hormone only was seen in 35.6%, β-luteinizing hormone only in 5.1%, β-follicle stimulating hormone and β-luteinizing hormone together in 22.3% and neither in 37.6%. The sensitivity, specificity, positive and negative predictive values of the transcription factors and hormones are shown in Fig. 5. Only 40.2% had a Ki67 labeling index <3% whereas 48.4% had a Ki67 labeling index of 3–5%, 9.7% had a Ki67 labeling index of 5–10% and 1.7% had a Ki67 labeling index ≥10%. Stains for p27 were negative in only 9.1% with an average score of 1.54 and FGFR4 was expressed in 84.3% with an average score of 1.64. Interestingly, among these tumors, keratin staining was negative in 37.1% of cases.

Pit-1 lineage-tumors

Pit-1 lineage-tumors numbered 316, representing 29.9% of the tumors. By definition these tumors all expressed Pit-1. No tumor was found that expressed growth hormone, prolactin or β-thyrotropin without Pit-1 but there were tumors that expressed only Pit-1 and no hormones of that lineage. The tumors were subclassified according to previously published criteria [10]. Somatotroph, mammosomatotroph or mixed tumors with predominant growth hormone reactivity numbered 167 (52.8%), lactotroph or acidophil stem cell tumors with predominant prolactin reactivity were 88 (27.8%), 7 were thyrotroph tumors (2.2%), poorly differentiated Pit-1 lineage tumors [12] numbered 44 (13.9%) and 10 tumors were classified as not-otherwise-specified (3.2%). The patients in this group included 132 males and 183 females with one lacking gender identification.

Pit-1 lineage tumors with predominant growth hormone immunoreactivity were classified as somatotroph, mammosomatotroph or mixed somatotroph-lactotroph differentiation ($n = 167$) included 60 densely granulated somatotroph tumors, 61 sparsely granulated somatotroph tumors, 3 somatotroph tumors with mixed densely and sparsely granulated components, 22 mammosomatotroph tumors, 2 plurihormonal tumors (growth hormone, prolactin and β-thyrotropin) with mammosomatotroph morphology, 6 unclassified somatotroph tumors and 3 unclassified tumors expressing both growth hormone and prolactin, and 10 tumors with two mixed distinct cell populations, either densely granulated or sparsely granulated somatotrophs admixed with sparsely granulated lactotrophs. These tumors usually presented with acromegaly with or without hyperprolactinemia. There were 96 women and 71 men and the mean age for this group overall was 45 years; interestingly patients with densely granulated somatotroph tumors had a mean age of 50 years whereas those with sparsely granulated somatotroph tumors had a mean age of 40 years. By definition, these tumors all exhibited nuclear positivity for Pit-1 and cytoplasmic positivity for growth hormone. Prolactin was found in mammosomatotroph or mixed tumors and the 2 plurihormonal tumors. ER positivity was identified in 35% and correlated with prolactin positivity. Only 1.96% of this tumor group was negative for keratins. All tumors classified as densely granulated somatotroph or mammosomatotroph tumors had perinuclear keratin staining that might have included focal fibrous bodies. In contrast, any lesion classified as a sparsely granulated

somatotroph tumor, whether pure or mixed, had predominant (>70% of the tumor cells), usually exclusive keratin positivity in the form of fibrous bodies. Immunoreactive α-subunit was found in 44% and α-human chorionic gonadotropin was found in 19%; these hormones correlated with densely granulated morphology, as well as perinuclear keratin, whereas sparsely granulated somatotroph tumors with fibrous bodies were generally negative for this marker. More than half of these tumors (54%) had a Ki67 labeling index <3%; when broken down into subgroups, only sparsely granulated somatotrophs had a majority with Ki67 labeling index ≥3%. Staining for p27 was positive in 68.5% of cases and FGFR4 was expressed in half of the tumors.

Pit-1 lineage tumors with predominant prolactin staining ($n = 88$) included 60 sparsely granulated lactotroph tumors, 1 densely granulated lactotroph tumor, 21 acidophil stem cell tumors and 6 lactotroph tumors not-otherwise-specified. All 60 sparsely granulated lactotroph tumors had a characteristic juxtanuclear pattern of prolactin immunoreactivity, allowing this diagnosis. Acidophil stem cell tumors were characterized by oncocytic cytoplasm and predominant prolactin immunoreactivity. The CAM5.2 stain was negative in 12.5% of these tumors; scattered fibrous bodies were seen in two thirds of acidophil stem cell tumors. Only 43.2% of these tumors had a Ki67 labeling index <3%; the highest labeling was seen in acidophil stem cell tumors where 78% had a Ki67 labeling index ≥3 and 50% were higher than 5%. Staining for p27 was positive in 84.8% of cases tested. FGFR4 was expressed in only 45.6% of cases examined.

This surgical series included 7 thyrotroph tumors (0.066%) that occurred in 4 females and 3 males with a median age of 41.7 years. These tumors were uniformly positive for PAS, Pit-1, β-thyrotropin, α-subunit and α-human chorionic gonadotropin; they were negative for SF-1 and ER. While one tumor had no data on CAM5.2, only one tumor was negative for CAM5.2 (16.7%), and no fibrous bodies were identified. Three had a Ki67 labeling index <3%, three had a Ki67 labeling index 3–5% and only 1 was >5%. All tumors expressed p27 and 4 expressed FGFR4.

There were 44 poorly differentiated tumors of Pit-1 lineage (4.2%), formerly classified as Silent Subtype 3 adenomas. The initial 31 cases have recently been reported [12]. These tumors all expressed Pit-1 and there was variable, often focal expression of growth hormone, prolactin, β-thyrotropin, α-subunit, and ER. Interestingly, 10 of 44 tumors (23%) had variable positivity for only one of the Pit-1 lineage specific hormones (growth hormone, prolactin and β-thyrotropin) and 6 of 44 (14%) poorly differentiated Pit-1 lineage tumors had no reactivity for these hormones. α-subunit was expressed in 5 of 9 monohormonal and 2 of 6 hormone-negative poorly differentiated Pit-1 lineage tumors. Keratin staining was negative in 11.4% and scattered fibrous bodies were identified in 29.5%. Only 16% of these tumors had a Ki67 labeling index <3%, the majority were 3–10% and in three tumors, the Ki67 labeling index was ≥10%. Staining for p27 was negative in 14.7% and FGFR4 was expressed in 74.2%.

The ten tumors classified as Pit-1 not-otherwise-specified were tumors that stained for Pit-1 but had either insufficient or inadequate tissue to carry out the requisite stains for characterization.

Corticotroph tumors

Corticotroph tumors represented 17.1% of the cohort ($n = 180$) occurring in 137 females and 43 males; the mean age was 48 years. They included 85 densely granulated corticotroph tumors, 52 sparsely granulated corticotroph tumors, 9 mixed corticotroph tumors (7 mixed densely and sparsely granulated corticotroph tumors, and 2 mixed densely granulated and Crooke cell tumors), 11 corticotroph tumors not-otherwise-specified and 23 Crooke cell tumors. Interestingly the mean age of patients with densely granulated tumors was 44 years, whereas that of patients with sparsely granulated tumors was 52 years. While females predominated in all groups, the lowest incidence in females was among Crooke cell tumors where only 64% were female. Selected corticotroph tumors that were subjected to Tpit immunohistochemistry showed nuclear reactivity. All corticotroph tumors were positive for keratins; the Crooke cell tumors had intense keratin reactivity filling the cytoplasm in a thick ring-like pattern. PAS positivity correlated with adrenocorticotropin reactivity that was present in all tumors. The Ki67 labeling index was <3% in 40% of these tumors. Among the 143 tested tumors, 54 (37.8%) were negative for p27. This represents the group with the most significant loss of p27 (Fig. 6).

A detailed breakdown of these tumors identified interesting correlations between structure and function with the exception of Crooke cell tumors that are not further evaluated in this report. Of the 157 corticotroph tumors, 91 were associated with adrenocorticotropin and cortisol hypersecretion, 54 were clinically nonfunctioning and the remainder had incomplete clinical data. Among the 85 densely granulated tumors, 68 were functioning, 15 were clinically silent and 2 had no clinical data. Among the 52 sparsely granulated tumors, 36 were clinically nonfunctioning, 10 were associated with hormone excess and 6 had incomplete clinical data. The 9 mixed tumors included 6 functioning and 3 nonfunctioning tumors. The 11 unclassified corticotroph tumors (not-otherwise-specified) had technical limitations that did not allow accurate subtyping; 7 of these were functional and 4 had incomplete data.

Staining for p27 has been reported to be reduced or lost mainly in corticotroph tumors [13–16] and this series

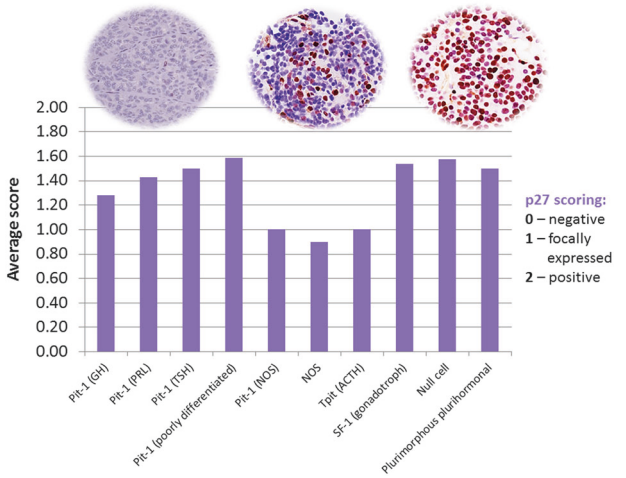

Fig. 6 Results of p27 staining in pituitary adenohypophysial neuroendocrine tumors. Staining for p27 was classified as negative (0, left photomicrograph), weak or focal (1), middle photomicrograph) or strong and diffuse (right photomicrograph). The graph illustrates the average staining for p27 in the various pituitary adenohypophysial tumor groups. (*GH* growth hormone, *PRL* prolactin, *TSH* thyrotropin, *NOS* not otherwise specified, *ACTH* adrenocorticotropin)

confirms that impression. Interestingly there was also a correlation of p27 loss with clinical evidence of glucocorticoid excess ($p = 0.0001$). Irrespective of the functional status, tumors with low p27 expression scores (scores 0 or 1) were enriched in densely granulated corticotroph tumors when compared with sparsely granulated corticotroph tumors
($p = 0.0001$). However, p27 scores did not correlate with tumor granulation phenotype (densely vs. sparsely granulated) within the functional ($p = 0.2651$) and nonfunctional ($p = 0.6468$) tumor groups, indicating that loss of p27 is unrelated to the granulation phenotype of corticotroph tumors.

Null cell tumors

Null cell tumors, defined as epithelial tumors of adenohypophysial cells that express no markers of cell differentiation based on lack of expression for adenohypophysial hormones and transcription factors, represented only 4.5% of this cohort ($n = 47$). They occurred in 28 females and 19 males, ages 24 to 85 years. Keratin was negative in 28.2% and six tumors expressed α-subunit alone. The Ki67 labeling index was <3% in 57% of these tumors, p27 was positive in all and FGFR4 was expressed by 80% of these tumors.

There were only 5 tumors that were "unusual plurihormonal" and therefore could not be classified into any specific category. These occurred in 3 men and 1 woman, and in one case, there was no gender provided. The average age was 50 years. All were positive for keratins, and 20% had fibrous bodies. They expressed unusual combinations of Pit-1, SF-1, and ER with various hormones from multiple lineages. The Ki67 was <3% in half and all were positive for p27.

Selected biomarker analysis

The analysis of *p27* (Fig. 6) is discussed above in each subgroup. While there was loss of this biomarker in small numbers of tumors in each major tumor group, the data indicate that p27 is of particular value in determining the clinical manifestations of corticotroph tumors.

FGFR4 staining

FGFR4 staining (Fig. 7) identified the highest incidence and levels of positivity in tumors that were gonadotrophs, null cell, poorly differentiated Pit-1 lineage tumors, and unusual plurihormonal tumors. There was a positive correlation between the tumor size and FGFR4 expression within the entire cohort ($p = 0.001$). Comparison of FGFR4 expression scores between tumors groups also showed statistical differences (Supplementary Table 2).

The Ki67 labeling indices

The Ki67 labeling indices were analyzed to determine the value of the proposed 3% cut-off to separate typical from atypical tumors [17]. In this series, Ki67 was greater than 3% in 60% of the tumors. Figure 8 illustrates the tumor types with the highest proliferative indices.

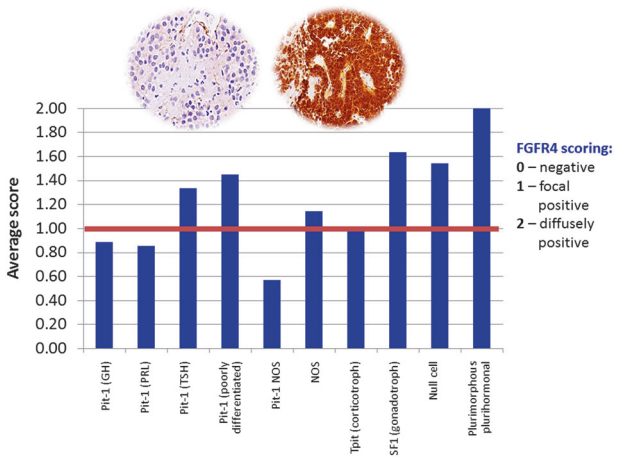

Fig. 7 Results of FGFR4 staining in pituitary adenohypophysial neuroendocrine tumors. Staining for FGFR4 was classified as negative (0, left photomicrograph), weak or focal (1) or strong and diffuse (right photomicrograph). The graph illustrates the average staining for FGFR4 in the various adenohypophysial tumor subtypes. (*GH* growth hormone, *PRL* prolactin, *TSH* thyrotropin, *NOS* not otherwise specified, *ACTH* adrenocorticotropin)

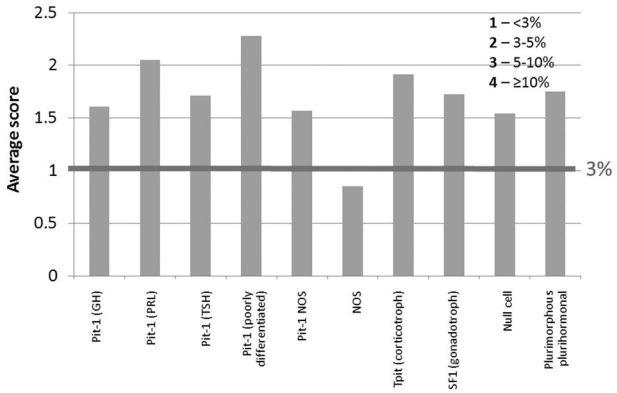

Fig. 8 Ki67 labeling index by tumor type in pituitary adenohypophysial neuroendocrine tumors. The Ki67 labeling indices were grouped as illustrated and the distribution in the various adenohypophysial tumor subtypes is shown in the graph. The lack of value of the proposed 3% cutoff is illustrated. (*GH* growth hormone, *PRL* prolactin, *TSH* thyrotropin, *NOS* not otherwise specified, *ACTH* adrenocorticotropin)

Discussion

This represents one of the largest surgical series of morphologically characterized pituitary tumors reported to date and the first to include the modern approach that applies the routine use of transcription factors for tumor classification in a large cohort.

The distribution of tumor types confirms previous suggestions that approximately 40% of surgically resected pituitary neuroendocrine tumors are clinically nonfunctioning tumors [3], the majority of which are of gonadotroph lineage [10, 18]. The value of transcription factors in separating gonadotrophs from null cell tumors has been reported [19], and the incidence of null cell tumors in this series was only 4.5%. This finding supports the prediction by Kovacs [20] in his initial 1980 paper that established the nomenclature and predicted that future studies would identify the cytogenesis of many null cell tumors [21]. As expected based on earlier studies [22, 23], the majority are gonadotrophs that can now be classified based on the expression of SF-1 [24] that is the most sensitive and specific biomarker of gonadotroph lineage as shown in this series. While the mean ages of patients with gonadotroph (58 years) and null cell (55 years) tumors is similar, interestingly males comprise the majority of patients with gonadotroph tumors (63%) whereas females predominate in the group of null cell tumors (60%).

The application of Pit-1 as a key determinant of lineage has also allowed clarification of a number of other tumor types. The Pit-1 family is the most diverse and complex in pituitary pathology. We have divided Pit-1 positive tumors into those with predominant growth hormone functionality, those most responsible for prolactin production, and thyrotroph tumors. The vast majority of these are associated with clinical features that correspond to this classification, but this category also has the highest incidence of plurihormonality. This is not surprising, since Pit-1 plays a role in the expression of growth hormone, prolactin and β-thyrotropin. An important finding is the high frequency of expression of α-subunit in these lesions, usually associated with growth hormone or β-thyrotropin.

The clinical importance of correct classification of adenohypophysial tumors with predominant growth hormone immunoreactivity has been recently emphasized [25], since morphologic classification correlates with differing clinical manifestations, identifies distinct pathogenetic mechanisms and predicts response to therapy. Here we document the importance of keratin staining to detect cytokeratins 8 and 18 for tumor classification. This distinguishes the sparsely granulated somatotroph tumors that have distinct imaging features [26–28] and less responsiveness to somatostatin analog therapy [10, 18, 29–38] from the group of densely granulated somatotroph and mammosomatotroph tumors that are usually detected when smaller, probably because of the higher secretory activity that results in florid symptomatology of acromegaly. Importantly, the significance of fibrous bodies must be interpreted with the understanding that occasional fibrous bodies may be seen in "intermediate type tumors" [39] that are functionally similar to densely granulated tumors, and only those with virtually all their keratin reactivity in fibrous bodies represent the true sparsely granulated somatotroph tumors. Interestingly, in this series we have also identified 3 somatotroph tumors with two discrete components, which we have classified as mixed densely and sparsely granulated somatotroph tumors. Other rare variants include mixed somatotroph and lactotroph tumors; we identified 6 tumors composed of admixed densely granulated somatotrophs and sparsely granulated lactotrophs, and 4 tumors with sparsely granulated somatotrophs admixed with sparsely granulated lactotrophs. There were only 3 tumors that were plurihormonal, composed of densely granulated cells that resembled mammosomatotrophs, but also expressed β-thyrotropin.

Lactotroph tumors are usually responsive to dopaminergic therapy and are therefore commonly treated medically. They are likely to be the most common adenohypophysial tumors [3–8] but this is not reflected in our series that is composed only of surgically-resected tumors. The majority of lactotroph tumors are composed of cells that resemble the normal sparsely granulated lactotroph; these tumors are also the most likely to respond to medical therapy. The disproportionate number of acidophil stem cell tumors in our series (21 of 88 tumors with predominant prolactin immunoreactivity) may be a reflection of the more aggressive nature of these tumors compared to lactotroph tumors that are usually responsive to medical therapies.

Thyrotroph tumors are known to be rare and comprise <1% of cases in our series. There were no unusual features in this category of tumors that have characteristic histology and immunoprofiles.

The category of poorly differentiated tumors of Pit-1 lineage was recently reviewed in detail [12]. The terminology for these lesions required change from "silent subtype 3" because these are not always silent and they are not a subtype of silent corticotroph tumor as initially thought. The recent WHO classification has proposed the term "plurihormonal Pit-1 positive adenoma" [40] but this is incorrect because they are also not always plurihormonal [41].

The category of corticotroph tumors provides interesting insight into the distribution of these lesions. Only 47% represent the typical basophilic densely granulated corticotroph tumors that are the classical source of pituitary Cushing's disease. The clinical differences between densely and sparsely granulated corticotroph tumors has been described as "small tumor, big Cushing" for the densely granulated and "big tumor, small Cushing" for the sparsely granulated tumors [42]. Corticotroph tumors can also be clinically silent [10, 18, 30, 43–48]. Our data confirm that loss of p27 nuclear staining was statistically more frequent in clinically functional corticotroph tumors than in clinically nonsecreting tumors. The existence of mixed tumors indicates a fluidity of these tumors and indeed, one case of a small densely granulated microadenoma associated with Cushing disease but was unresectable recurred as a sparsely granulated macrotumor with the development of Nelson syndrome. The presence of a large number of unclassified tumors [11] is also not surprising, since these lesions tend to be small and the tiny specimens that may also have cautery artifact are occasionally too small to undergo all the required testing. The identification of 23 Crooke cell tumors in this series is unexpected, since these are thought to be rare.

Among the key findings is the surprising number of keratin-negative tumors. While all corticotroph and plurihormal tumors were CAM5.2 positive, other well characterized tumor groups showed variable negativity rates with the highest negativity in gonadotroph (37.1%) and null cell tumors (28.2%). This represents a potential pitfall in diagnostic pathology, since the differential diagnosis includes olfactory neuroblastoma or sinonasal neuroendocrine tumors that may be mistakenly diagnosed in the same clinical setting [49], and primary sellar or parasellar paragangliomas that can mimic adenohypophysial tumors. Lack of keratin staining has been considered to be reliable in the diagnosis of these tumors but our study indicates that this alone is insufficient; the application of additional pituitary biomarkers, especially transcription factors, is required to arrive at the correct diagnosis.

The additional ancillary markers in this study included p27 and FGFR4. As previously reported [13], p27 was lost mainly in corticotroph lesions. We are the first to identify a correlation between loss of p27 and clinical functionality of these tumors; our data suggest that the loss of p27 correlates with elevated glucocorticoids levels in patients with corticotroph tumors. This may be explained by the role of glucocorticoids in p27 degradation that is dependent on Skp2 [50]. Loss of p27 may be of value in identifying patients with multiple endocrine neoplasia type 4 (MEN-4) [51–53]; however this appears to not be of value in corticotroph tumors.

FGFR4 is a growth factor that has been identified in pituitary tumors [54] and implicated in adenohypophysial cell tumorigenesis [10, 55]. In some instances, an FGFR4 polymorphism may modulate the clinical behavior of pituitary tumors [56, 57].

Tumors with a Ki67 labeling index ≥3% comprised 60% of this cohort. While the fourth edition of the WHO Classification of Tumors of Endocrine Organs abandoned the concept of the atypical pituitary adenoma [40], the third edition of the WHO classification that was in use during the period reported categorized such tumors as "atypical" [17]. Our results support the lack of value of that categorization. Indeed, the best predictor of aggressive behavior of pituitary adenohypophysial tumors is not this proliferation marker; instead the tumor classification and radiological extent of disease that will determine the outcome of surgical resection are the true prognostic classifiers [58].

In conclusion, we report one of the largest series of morphologically characterized surgically resected pituitary adenohypophysial tumors. We provide data based on the routine use of transcription factors for tumor classification. The data provide the most comprehensive epidemiologic information for these tumors and the basis for clinicopathologic correlations that are helpful for prognostic and predictive patient management.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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