Endocrine (2012) 42:18–28
DOI 10.1007/s12020-012-9660-9

REVIEW

Clinical implications of growth hormone–secreting tumor subtypes

Katja Kiseljak-Vassiliades · Shibana Shafi · Janice M. Kerr · Tzu L. Phang · B. K. Kleinschmidt-DeMasters · Margaret E. Wierman

Received: 29 January 2012 / Accepted: 9 March 2012 / Published online: 21 March 2012

Abstract Growth hormone (GH) pituitary tumors are almost always benign adenomas, yet are associated with significant morbidity and mortality. Surgical and medical responses of GH tumors are often incomplete, and therefore predictors of residual or recurrent disease are needed. Clinical features, including patient gender, age or size of adenoma, have proven to be unreliable predictors of recurrence. Differing clinical behavior between the two GH tumor subtypes, sparsely granulated (SG) versus densely granulated (DG), has been reported, but has not been used routinely in clinical management. SG tumors are more common in younger patients (<50 years), and are usually larger tumors. SG tumors have been reported to be less responsive to somatostatin analogs (SSA) than DG tumors. The mechanisms underlying these potential differences in tumor behavior, however, are poorly defined. Subsets (up to 50 %) of DG adenomas harbor a *gsp* mutation that can activate cAMP that provides a theoretical intracellular target for somatostatin therapy. In contrast, some SG tumors have reduced somatostatin receptor expression and mutations in the extracellular domain of the GH receptor that may contribute to SSA resistance. While DG versus SG growth hormone adenomas are readily distinguished by immunohistochemistry, other less common GH adenoma variants still require electron microscopy (EM) for confident subclassification. Whether these less common variants possess unique clinical features is unknown. Research is needed to identify clinically relevant biomarkers of GH pituitary tumors that predict risk of recurrence and response to medical therapy.

Keywords Pituitary adenoma · Growth hormone tumor · Acromegaly · Densely granulated adenoma · Sparsely granulated adenoma

K. Kiseljak-Vassiliades · S. Shafi · J. M. Kerr · M. E. Wierman
Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

K. Kiseljak-Vassiliades · M. E. Wierman (✉)
Research Service Veterans Affairs Medical Center, Denver, CO 80220, USA
e-mail: margaret.wierman@ucdenver.edu

T. L. Phang
Department of Critical Care and Pulmonary, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

B. K. Kleinschmidt-DeMasters
Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

B. K. Kleinschmidt-DeMasters
Department of Neurology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

B. K. Kleinschmidt-DeMasters
Department of Neurosurgery, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

Introduction

Acromegaly is a disorder of abnormal skeletal and visceral tissue growth triggered by oversecretion of growth hormone (GH). In the vast majority of cases, acromegaly is caused by a GH pituitary adenoma, with an incidence of five per million [1, 2]. Although most often benign, these tumors are associated with significant somatic, metabolic and cardiovascular complications. GH tumors are detected as macroadenomas (tumors ≥1 cm) in 65 % of cases and often produce local compression of adjacent sellar structures, with associated visual impairment and pituitary hormone deficiencies [3]. Chronically elevated serum GH

levels lead to a 10-year reduction in life expectancy due to cardio- and cerebrovascular, respiratory, metabolic and endocrine complications [2].

The goal of acromegaly treatment is biochemical normalization of IGF-1 and GH levels, appropriate for age and gender [4]. Most guidelines suggest surgical resection as first-line therapy for GH tumors [5]. Unfortunately, because most tumors are large and invasive, less than 50 % of patients achieve long-term biochemical control postoperatively with surgery alone, even in experienced clinical centers [3, 6]. Medical therapy with somatostatin analogs (SSA) or more recently a GH antagonist (pegvisomant) is indicated for patients with persistent disease [7–9]. Dopamine agonists have a limited role in the management of acromegaly [5]. The availability of different medical strategies has resulted in uncertainty and complacency about treatment implementation and optimization [10]. Specifically, ~40–50 % of acromegalic patients, treated with SSAs, have uncontrolled progressive disease despite surgery and medical therapy [11]. In addition, despite the initial excellent response rate to the alternative therapy, pegvisomant, more recent experience suggests IGF-1 level normalization in <70 % of patients, at least some of which may have been attributed to inadequate dosing of the medication [12]. Radiotherapy is often required as adjunctive therapy, but requires a long latency period to achieve remission (50 % at 10 years) [2]. Thus, improved treatment options are still needed for a large subset of patients.

Although some investigators have used the clinicopathological parameters of patient gender, age, adenoma size and Ki-67 labeling as prognostic predictors of GH-secreting adenoma behavior, results in the literature have been conflicting. Several studies reported an inverse relationship between patient age and likelihood of tumor persistence [13, 14]. Larger tumors have been frequently associated with invasiveness and poorer response to therapy [3, 15]. In a retrospective study of 254 patients, univariate analysis showed that younger age and larger tumors were predictive of disease persistence; however, in multivariate analysis, none of these variables achieved statistical significance [16]. A recent meta-analysis of nine studies concluded that tumor size and invasion were not definite prognostic factors in acromegaly [17], although some of these studies were not sufficiently powered to directly address this question. On pathological aspect, Fusco and coworkers examined prognostic significance of nuclear antigen Ki-67 and reported that Ki-67 labeling was significantly lower in cured patient group ($n = 28/68$) with mean value of 1.5 ± 1.2 %, then in group with persistent disease ($n = 13/68$) with mean value of 1.8 ± 1.5 % ($p < 0.01$) [18]. This was, however, in contrast to others who failed to find such a correlation [19, 20]. No association was reported between high Ki-67 levels and MGMT (*O*-6 methylguanine DNA methyl transferase) immunoreactivity. MGMT is a recently identified marker that has been inversely correlated to GH tumor response to temozolomide, a novel treatment for aggressive pituitary adenomas and carcinomas [21].

Pathologists have further defined GH tumor histology, by densely (DG) and sparsely granulated (SG) subtypes [22], which have aided the ability of endocrinologists to predict tumor activity. Histological features of DG- and SG-GH adenomas are contrasted in Figs. 1 and 2, respectively. Hematoxylin–eosin staining of the two subtypes is depicted in Fig. 1a (DG tumor) and Fig. 2a (SG tumor). Histology is an important predictor of persistent disease and has been correlated with responsiveness to medical therapy with somatostatin analogs, with SG adenomas showing lower responsiveness than DG adenomas [23]. No data are available as to whether GH histologic subtype predicts response to pegvisomant, the GH receptor antagonist. The issue of GH subtyping has been greatly facilitated by the use of immunohistochemistry for CAM 5.2, an antibody that recognizes the cytoplasmic keratin filaments present as fibrous bodies in SG adenomas, but not DG (see Figs. 2c and 1d, respectively) [24]. Immunohistochemistry has largely eliminated the need for electron microscopy (EM) in this distinction, but it is still required to subclassify less common GH variants [24, 25]. Importantly, however, even with publications detailing the importance of sub-classifying GH adenomas as SG versus DG on the basis of immunohistochemistry, the sub-classification is not often used clinically to decide which medical therapy to pursue.

This review outlines the current data on the correlation of histology of SG- and DG-GH tumor subtypes, to clinical and biochemical outcomes and characterize their value as prognostic predictors in acromegalic patients. At present, these features, although promising, are inadequate and future research is needed to determine the pathophysiology of these tumors, biomarkers of disease activity and progression and predictors of response to somatostatin analogs (SSA) compared with GH antagonist therapy for patients with residual or recurrent disease.

DG versus SG GH tumors—a historical perspective

Advances in pathological characterization of GH tumors using EM imaging initially led to the immunohistochemical division of GH tumors into two subtypes with distinct ultrastructure features; densely and sparsely granulated adenomas [26, 27]. While DG tumors display well-developed organelles and abundant large secretory granules on electron microscopy, SG-GH tumors manifest

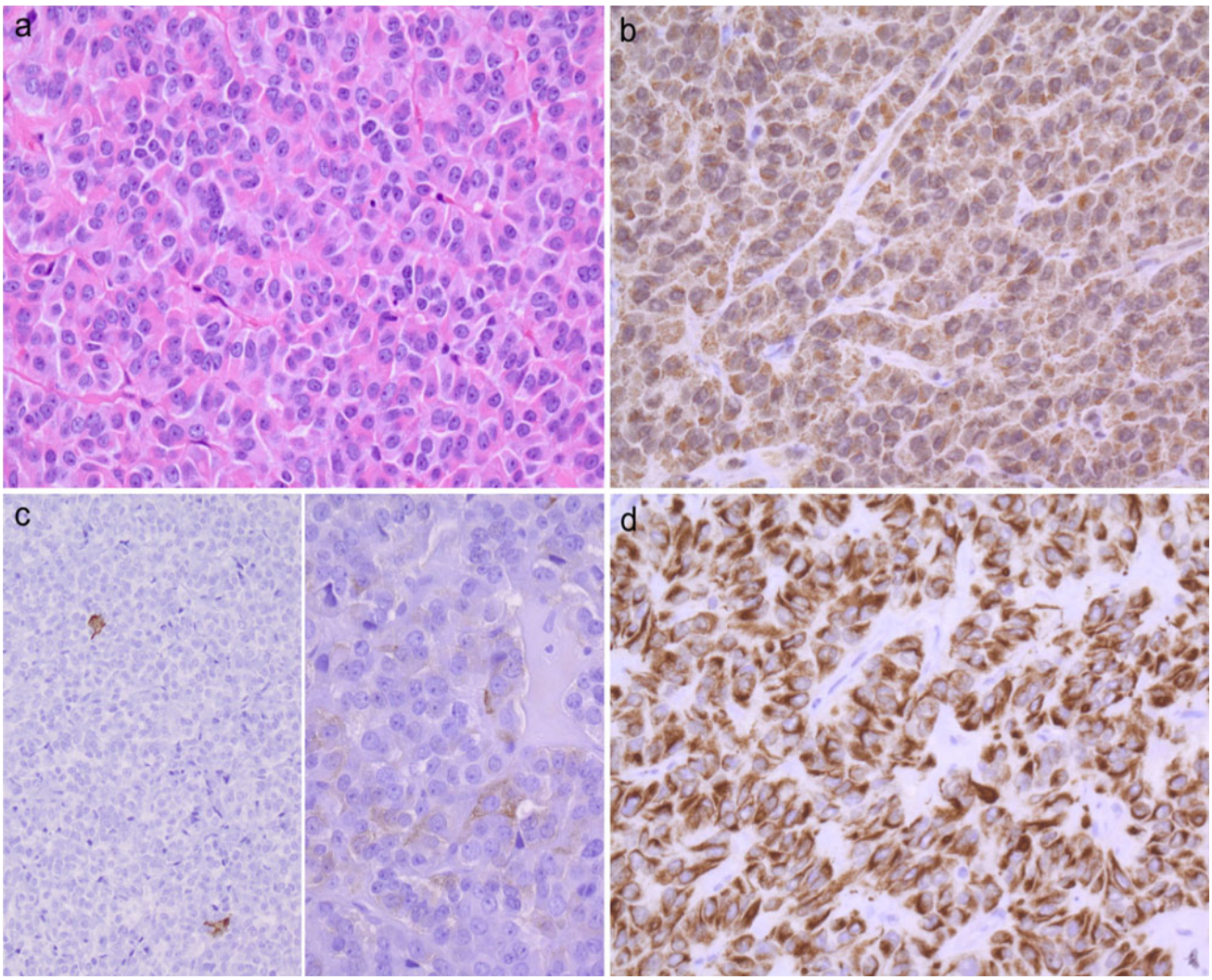

Fig. 1 Densely granulated growth hormone cell adenoma. **a** DG-GH adenomas show a monomorphic cell population with abundant eosinophilic cytoplasm on hematoxylin and eosin stain. **b** There is diffuse immunoreactivity for growth hormone throughout the majority of cells in the adenoma. **c** DG-GH adenomas may also show focal immunoreactivity for prolactin (**c**, *left*), or alpha subunit (**c**, *right*). **d** Keratins are distributed throughout the cytoplasm, as seen on Cam5.2 immunohistochemistry

sparse neurosecretory granules (see Fig. 2d). Sano and coworkers subsequently used a differential cytokeratin expression pattern (with CAM 5.2 keratin stain) as a surrogate marker between DG and SG GH tumors [28]. Cytokeratin immunostaining under light microscopy proved to be an adequate, more cost-effective substitute for EM with DG showing perinuclear or non-dot pattern and SG displaying dot cytokeratin pattern [24]. Fibrous bodies, found specifically in SG tumors, form spherical aggregates of cytokeratin containing filaments in the region of Golgi membrane and smooth-surfaced endoplasmic reticulum (see Fig. 2c) while these are not present on CAM 5.2 staining in DG tumors (see Fig. 1d). Besides its structural role, cytokeratin has an important role in many cellular functions, including protein synthesis, membrane traffic and signaling, and its disruptions could result to abnormal cellular function [29]. The implication of variable cytokeratin patterns in morphologically distinct GH tumor subtypes is unknown.

Most GH adenomas are either DG or SG, but unfortunately, the subclassification is not quite as simple. GH-secreting adenomas often additionally display immunoreactivity for other anterior pituitary hormone types, usually prolactin (PRL) followed by the alpha subunit (α-subunit) of glycoprotein hormones; less commonly the beta-subunit (β-subunit) of follicle-stimulating hormone (FSH), luteinizing hormone (LH) or even thyroid-stimulating hormone (TSH) is also detected [24, 25]. Figure 1c depicts PRL (*left*) and alpha subunit (*right*) immunostaining in DG tumor.

Regarding plurihormonality, most studies demonstrate that GH, TSH and α-subunit immunoreactivity are consistently weaker in SG than in DG-GH pituitary tumors

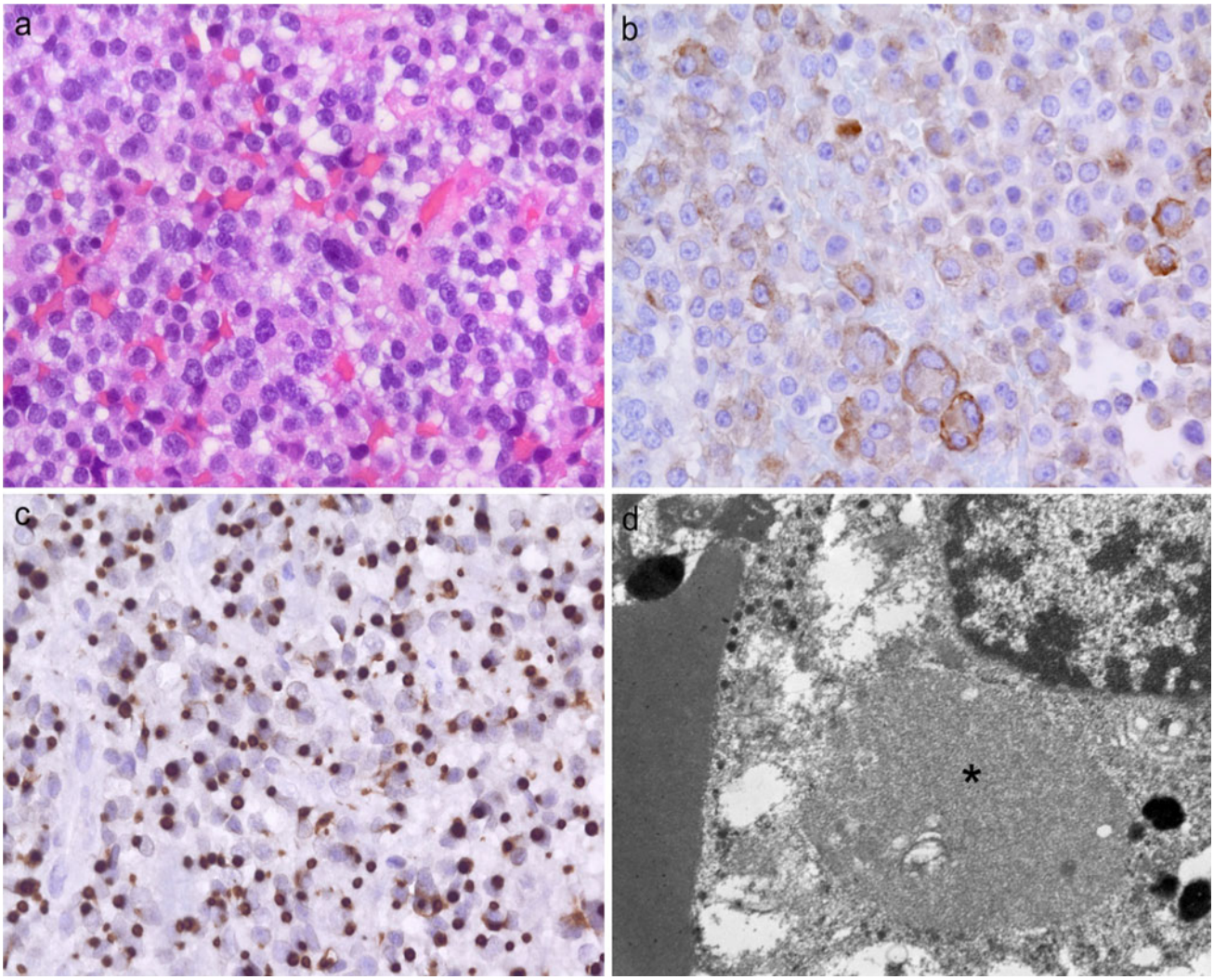

Fig. 2 Sparsely granulated growth hormone adenoma. **a** SG-GH shows a relatively monomorphic population of cells on hematoxylin and eosin stain (H&E), although occasional cells may show nuclear enlargement on H&E. **b** Immunostaining for growth hormone is usually patchy and sparser, as reflected in the name. **c** Unlike DG-GH, SG-GH shows dot-like keratin immunoreactivity in the cytoplasm on Cam5.2 immunostaining, paralleling the presence of fibrous bodies. **d** Fibrous bodies on electron microscopy consist of rounded collections of filaments (*) located adjacent to the nucleus (*upper right*)

[28, 30–33], although a recent study did not detect a difference in GH and TSH staining between the tumor subtypes [34]. The PRL immunoreactivity has been variable [28, 30–34]. Figures 1b and 2b illustrate GH immunostaining in DG compared with SG, respectively, with GH staining being more prominent in DG compared with SG tumors. An obvious weakness of immunohistochemistry is that differing antibody sources, retrieval methods, variable tissue fixation and even inter-laboratory differences in technique can affect the intensity of the hormonal staining pattern. Despite showing immunoreactivity for various additional hormones, these tumors usually do not show significant serum elevations or clinical manifestations related to these additional hormones, with the exception of PRL. Lopes reported that 50.8 % of patients with surgically removed GH adenomas presented with signs and symptoms of both acromegaly and hyperprolactinemia [25, 35].

Three types of mixed GH-/PRL-secreting adenomas have been described [24], all of which require EM for subclassification: mixed GH-PRL adenomas, mammosomatotroph cell adenomas (single-cell type similar to DG adenomas secreting both PRL and GH in the same cell) and acidophilic stem cell adenomas (single immature cell type with features of SG and PRL cells, with aggressive behavior and oncocytic, mitochondrion-rich cytoplasm) [24, 25]. The last two are quite rare (<2 % of all adenomas collectively), although mammosomatotroph cell adenomas are cited as constituting 8 % of all GH adenomas [25, 36]. However, mixed GH-PRL adenomas due to a dual-cell

population are fairly common and can consist of DG-PRL or SG-PRL combinations [25]. Whether these dual-cell population, mixed GH-PRL adenomas have further differing behavior or patient demographics from pure DG and pure SG adenomas has not been investigated.

In addition, some authors have further suggested that classification of pure DG versus pure SG GH adenomas based on cytokeratin (CAM 5.2) immunoreactivity is too simplistic. In a detailed study of 104 GH adenomas, Obari and coworkers divided cases into those 47 pure DG type adenomas with perinuclear cytokeratin immunoreactivity, 31 pure SG-type adenomas with dot-like deposits of globular cytokeratin immunoreactivity and 26 adenomas in which a "transitional" pattern of cytokeratin immunostaining could be identified [31]. The latter were described as having "halfway-formed or non-globular dots, occasionally accompanied by complete or incomplete ring-like immunoreactivity" [31]. In their classification schema, intermediate-type adenomas with "transitional" immunostaining pattern for cytoplasmic keratin behaved identically to DG GH adenomas [31]. These data do raise the possibility than differences between various pathologists in how they interpret such "transitional" cytokeratin immunostaining patterns in GH adenomas may alter the subclassification into GH adenoma subtypes on the basis of CAM 5.2 immunohistochemistry.

Correlation to clinical presentation and subtype of GH tumor

Since the initial description of histologic difference in GH tumor subtypes, there has been an interest in correlating specific histological patterns to clinical, hormonal and radiological presentation [28]. The studies are summarized in Table 1.

Patient characteristics

The literature correlating GH tumor subtypes to age and gender is limited to five studies with a total of 420 patients. A study of 104 Japanese patients with GH tumors, 31 SG-type tumors, 47 DG-type adenomas and 26 with mixed pattern found that mean age was lower (43.6 ± 11.1 vs. 49.6 ± 13.8 years, $p < 0.05$) in patients harboring SG compared with DG tumors [31]. Similar findings were reported in three other studies: Bando et al. reported 21 patients with mean age 39 ± 4.2 versus 47.4 ± 14.6 years, Mazal et al. described 76 patients with mean age 38.66 versus 46.36 years and Bakhtiar et al. presented 141 patients with mean age 45.0 ± 16.1 versus 50.1 ± 12.6 years for SG compared with DG tumors, respectively [30, 32, 34]. With respect to gender, a retrospective study of 31 GH tumors concluded that SG tumors were more frequent in females: 23 DG (18 male and 5 female) and 8 SG (1 male and 7 female) [33]. Subsequently, three larger studies, however, did not substantiate these initial reports, detecting no correlation between gender and morphological variants of GH pituitary tumors [31, 32, 34]. Recently, a study from Norway found no correlation between GH adenoma granulation pattern and age or gender, but this study was limited by a small number of SG tumors ($n = 10$) available for analysis [37]. In summary, taken together, these data support a correlation between GH subtype and age, but not gender, with SG-GH tumors being more common in the young.

Correlation of histology with tumor size and invasiveness

Although division of GH tumors by size into microadenoma (<1 cm) and macroadenoma (≥1 cm) has been widely accepted, further correlation of tumor-staging approaches with clinical outcomes is lacking. Radiographic imaging has limited value in staging pituitary tumors, but the investigators have used Wilson's modification of Hardy's criteria and Knosp classification to stage these tumors. Wilson modification of Hardy's radiographic categorization is defined as: stage-I tumor <1 cm, stage-II tumor >1 cm, but intrasellar/slightly suprasellar, stage-III invasion of sella and stage-IV diffuse destruction of sella [38]. In addition, a Knosp classification (using internal carotid arteries as landmarks for invasiveness) has been used [39].

Using Wilson's modification of Hardy's criteria, Bando and coworkers [38] demonstrated that tumors stage III and IV were seen in 86 % ($n = 6/7$ tumors) of patients with SG and 31 % ($n = 4/13$ tumors) of patients with DG-GH tumors [30]. Using the same criteria, Yamada and coworkers reported 87 % ($n = 7/8$ tumors) of SG tumors were stage III or IV tumors compared with DG of 22 % ($n = 5/23$ tumors) [33]. In contrast, Mazal and coworkers did not find a significant correlation between GH tumor subtypes, but reported more frequent suprasellar extension (84 %; $n = 24/29$ tumors) and cavernous sinus infiltration (75 %; $n = 22/29$ tumors) in SG tumors compared with DG tumors (33 %; $n = 16/47$ tumors and 26 %; $n = 12/47$, respectively) [32]. A more recent report of 104 patients confirmed that SG-type tumors are larger and more invasive, as well as more advanced using the Knosp classification [31]. Thus, SG tumors tend to be larger and more invasive in comparison to DG adenomas. Hagiwara and coworkers also explored MRI characteristics of GH compared with non-GH tumors and found that on T2-weighted MRI images signal hypointensity was more commonly seen in GH adenomas ($n = 16/40$ [40 %]); $p < 0.001$) with finding been exclusive to DG-GH tumors [40]. A more

Table 1 Studies correlating clinical predictors and two growth hormone tumors subtypes, densely granulated (DG) and sparsely granulated (SG)

Studies	Age mean (years)	Gender (M/F)	Tumor size/invasive	Immunostaining	Hormone levels basal
Ezzat et al. [55]				DG—extensive immunoreactivity	DG—GH: 49 ± 15 μg/L
				SG—variable staining	IGF-I: 7443 ± 1117 U/L
					SG-GH: 43 ± 10 μg/L
					IGF-I 8979 ± 1555U/L
Yamada et al. [28]	DG—47.4 (26–66)	DG—18/5	DG—22 % III and IV[a]	DG—GH diffusely present; PRL 43 %; TSH-β 26 %; α-subunit 87 %	DG—GH: 44 ± 38 ng/mL
	SG—36.6 (22–46)	SG—1/7	SG—87 % III and IV[a]	SG—GH variable present; PRL, TSH-β and α-subunit all negative	IGF-I: 3.3 ± 1.0 U/mL
					SG—GH: 71 ± 52 ng/mL
					IGF-I: 3.7 ± 0.7 U/mL
Bakhtiar et al. [29]	DG—50.1 ± 12.6	DG—48.2 %/51.8 %	DG—17.5 ± 8.9 mm[b]	DG—70.2 ± 35.3 E-cadherin	DG—GH: 46.2 ± 130.5 ng/mL
	SG—45.0 ± 16.1	SG—36.7 %/63.3 %	SG—23.5 ± 11.5 mm[b]	SG—9.9 ± 25.1 E-cadherin	SG—GH: 115.7 ± 326.9 ng/mL
			No significant difference in invasiveness	GH, PRL, TSH-β positivity with no significant difference	
Bando et al. [25]	DG—47.4 ± 14.6	DG—4/9	DG—31 % III and IV[a]	DG—GH diffusely present, α-subunit 85 %	DG—GH: 33.3 ng/mL (mean)
	SG—39.5 ± 4	SG—2/5	SG—86 % III and IV[a]	SG—GH variable present, α-subunit 14 %	SG—GH: 59.9 ng/mL (mean)
Obari et al. [26]	DG—49.6 ± 13.8		DG—70 % II, III and IV[a]	DG—GH 87 %; α-subunit 43 %; PRL 49 %; TSH-β 13 %; E-cadherin 90 %	DG—GH: 59.4 ± 82.9 ng/mL
	SG—43.6 ± 11.1		SG—94 % II, III and IV[a]	SG—GH 29 %; PRL 29 %; no α-subunit or TSH-β; E-cadherin 5 %	SG—GH: 40.6 ± 43.9 ng/mL
			DG—38 % invasive		
			SG—65 % invasive		
Mazal et al. [27]	DG—46.4 (14–70)	DG—57/43	DG—33.3 % SE[c]	DG—GH diffusely present; PRL 79 %; TSH-β 53 %; α-subunit 28 %	
	SG—38.7 (18–65)	SG—69/31	26.1 % CI[d]	SG—GH variable present; PRL 48 %, TSH- β and α-subunit all negative	
			SG—84 % SE[c]		
			75 % CI[d]		
Fougner et al. [32]	DG—56 (46–60)	DG—3/7	No significant difference in tumor size and invasiveness	DG—0.351 E-cadherin ratio	DG—GH: 27.1 (10.5–47.5) mU/L
	SG—46 (40–52)	SG—13/18		SG—0.013 E-cadherin ratio	IGF: 89 (72.2–109) nmol/L
					SG—GH: 33.1 (10.1–96) mU/L
					IGF: 81.8 (47.5–109) nmol/L

[a] Hardy's criteria

[b] Mean maximal tumor diameter

[c] Suprasellar extension

[d] Cavernous infiltration

recent study, also looking at the correlation of MRI signal intensity to GH subtypes and response to somatostatin analogs, as primary therapy, reported that 100 % ($n = 8/8$) of SG were classified as hyperintense and 42 % ($n = 5/12$) of DG as hypointense on T2-weighted images ($p = 0.001$) [41]. The authors also reported that mean IGF-1 reduction, in 6-months follow up after treatment with somatostatin analog, was 51 (49–70) % in hypointese versus 13 (5–42) % in hyperdense group. The limitation of the study, however, is that in up to 20 % ($n = 10/45$) of MRI, there was discordance between the evaluating radiologists [41].

Correlation of histology and hormone levels

Several surgical studies, as well as a recent meta-analysis, found that preoperative GH level was an important outcome predictor in rates of remission in acromegaly [15–17, 35, 42, 43]. When correlating, preoperative GH levels and histological subtypes of GH tumors, two studies noted a trend that SG tumors are associated with lower basal GH levels. Obari and coworkers reported that mean value of basal GH levels in SG cases (40.6 ± 43.9 ng/mL) was lower than those of DG cases (59.4 ± 121.3 ng/mL) ($p > 0.05$) [31]. Bakhtiar et al. found that GH index (GH secretion per tumor volume) tended to be lower in SG (17.3 ± 22.2 ng/mL per cm^3) than in DG tumors (23.2 ± 39.1 ng/mL per cm^3) ($p = 0.145$) [34]. Others, however, did not find association between basal GH levels and histological subtype of GH adenoma [30, 33]. No correlation of histologic subtype with IGF-1 levels has been performed. Although uncommonly used in practice, GH response to TRH and GHRH stimulation were evaluated with respect to GH tumor type. In the postoperative period, patients with DG manifested higher TRH- and GHRH-stimulated GH levels than patients with SG tumor [30, 33, 34]. These observations, however, were not validated by later reports [44–48]. A major disadvantage of these dynamic tests is the lack of standard definition of an abnormal GH stimulation response, with abnormal ranging from 50 to 200 % of basal GH levels [30, 33, 34, 44, 49], as well as the lack of availability of TRH and GHRH reagents clinically.

Investigators have also explored various histological parameters associated with tumor aggressiveness and while most found no association between MVD (microvascular density) and p53 expression and GH tumor types [34], MIB-1 index, an indicator of proliferation, was higher in SG adenoma than in DG adenomas (4.23 vs. 2.07 %, $p < 0.0001$) [32]. DG tumors also have higher number of cells reactive for E-cadherin and β-catenin, suggesting a more differentiated phenotype [31, 34, 37, 50, 51]. E-cadherin and β-catenin form complexes serving as adhesive links between the cells and playing an important role in cell polarity across the epithelial sheet. Impairment of E-cadherin and β-catenin and subsequently weakening of the adherens junction components have been demonstrated to play an important role in induction of epithelial–mesenchymal transition (EMT) during tumorigenesis [52–54]. The exact role and implication of loss of E-cadherin and β-catenin expression in SG-GH pituitary tumors, however, has not been elucidated.

Pathophysiological differences underlying DG and SG tumors

Alterations in the normal signaling from GH-releasing hormone (GHRH) activating the GH receptor and ultimately –cAMP axis has been implicated in GH tumorigenesis [55]. On binding to its G-protein coupled receptor, GHRH signals via adenylate cyclase (AC) and cAMP to induce CREB (cAMP response element-binding protein) mediated transcription of GH. The potential molecular mechanisms identified in DG and SG pituitary tumors are depicted in Fig. 3. An activating mutation in G protein subunit α (*gsp*) leads to constitutively active cAMP resulting in high GH and is a putative cause of GH tumors and acromegaly. Shortly after altered *gsp* and adenylate cyclase (AC) activity was reported in human GH tumors [55, 56], Spada and coworkers examined the correlation of the presence of a constitutively active *gsp* mutation and GH tumor subtype [57]. In this 9-year retrospective study, only 33 % of all GH tumors ($n = 26/80$) had the gsp mutation resulting in constitutively active AC. Intriguingly, this alteration was more frequently observed in DG than in SG tumors [57]. This observation was recently reexamined by Bakhtiar and coworkers who found that 58 % of all GH tumors ($n = 25/43$) had *gsp* mutations. Although the mutation was detected in 66 % of DG ($n = 20/30$) tumors, in contrast to Spada and coworkers [57], 38 % of SG ($n = 5/13$) also harbored a deregulated *gsp* [34]. Both groups observed that tumors carrying altered *gsp* were smaller in size and had significantly higher GH level per volume of tumor compared with tumors without the mutation. When exposed to octreotide, 44 % of patients ($n = 4/9$) harboring the mutation and 18 % of patients ($n = 2/11$) without the mutation displayed a treatment response defined as a 90 % or greater decrease in the GH level from the baseline during testing [34]. Considering that adenylate cyclase is an intracellular target for somatostatin analog inhibition, it is surprising that less than half of patients responded to octreotide, suggesting that the *gsp* mutation is not the driving force in tumorigenesis and that other abnormalities exist within these tumors that remain to be elucidated. The concept that *gsp* mutations alone are insufficient for neoplastic transformation is

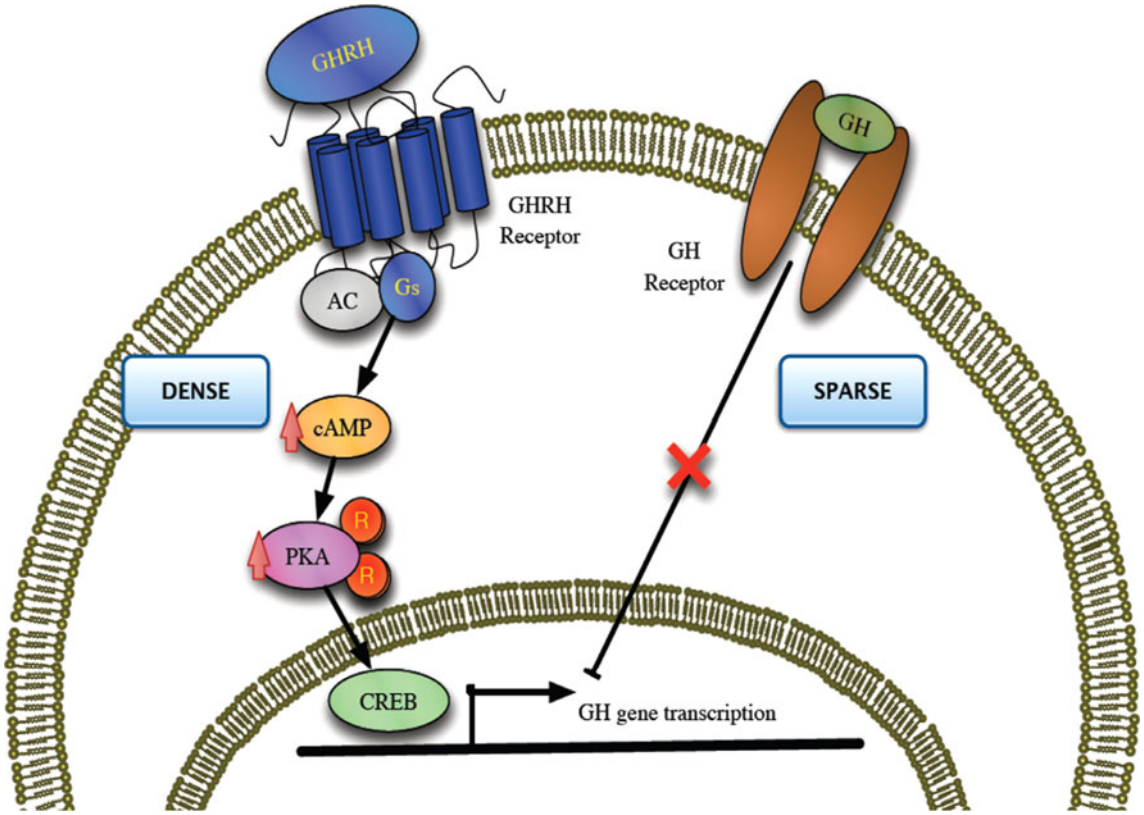

Fig. 3 Molecular defects in GH adenoma subtypes postulated to impact clinical presentation and prognosis. *Left* DG growth hormone tumors may have an activating mutation in G protein subunit α (*gsp*), leading to constitutively active cAMP and PKA and resulting in high GH as a putative cause of excess growth hormone and acromegaly. *Right* Some SG growth hormone tumors have mutations in the extracellular domain of the growth hormone receptor leading to disruption of GH auto-feedback signaling that may contribute to their pathogenesis

supported by transgenic mouse models of GH overexpression and McCune-Albright patients; both of which frequently show GH hyperplasia on histology, without evidence of GH adenomas [58]. Interestingly, a recent study, limited by small number of SG patients, reported no correlation between *gsp* mutation and GH adenoma morphology (*gsp* mutation present in 43 % [$n = 13/30$] of DG adenomas and 33 % [$n = 3/9$] of SG tumors) [37].

Asa and coworkers suggested that mutations within the GH receptor (GHR) may play a role in the pathogenesis of SG tumors via impaired GH auto-feedback. A heterozygous exon 4 GH receptor mutation was detected in 6 of 14 SG tumors, but none of the 12 DG tumors [59]. In vitro studies demonstrated that this mutation was associated with impaired GH receptor processing, ligand binding and signaling. In addition, they showed that pegvisomant action to antagonize GH in primary cultures of human DG growth hormone tumors led to fibrous body formation, similar to the SG phenotype. The authors hypothesized that altered GH binding contributes SG tumorigenesis. Others did not detect mutated GH receptors in 18 GH tumors, although the distinction between SG and DG tumors was not defined [60]. Importantly, transgenic GHR knock-out mice developed somatotroph hyperplasia without frequent evidence of GH adenomas, suggesting an alternate mechanism for SG tumor pathogenesis [61].

GH adenoma subtypes and treatment response

Despite the surgical advances and availability of medical therapy with somatostatin analogs or a competitive GH antagonist, 40–50 % of acromegalic patients are insufficiently treated and have persistent or recurrent disease [11]. Despite numerous publications studying the efficacy of SSA in normalizing GH/IGF-1 levels and decreasing tumor size, these studies are heterogenous regarding patient selection, definition of response, varying criteria for GH cure over the years and treatment duration. In addition, the majority of studies have not distinguished GH responsiveness by tumor subtype.

Bando and coworkers examined the ability of a single dose of octreotide acetate to suppress GH levels for 8 h and demonstrated that patients with DG tumors had significantly better GH suppression than patients with SG tumors (nadir/basal ratio 13.5 ± 5.8 % in DG vs. 42.9 ± 6.6 % in

SG), suggesting they would be more responsive to long-term SSA therapy [30]. Subsequently, Ezzat and coworkers examined the relationship between GH tumor subtype and response to octreotide therapy in 68 acromegalic patients [62]. Patients were characterized as those who received octreotide preoperatively and those who received surgery alone. GH levels were assessed at 1 month following the procedure in surgery alone group; and after 4 months of octreotide therapy in combination group. In the latter group, GH levels were measured for 4 h following octreotide administration. While there was no difference in basal GH levels after surgery or after octreotide followed by surgery, patients harboring DG tumors showed greater GH suppression after preoperative octreotide compared with those with SG tumors; GH was reduced to 30 and 70 % of baseline level, respectively. Interestingly, however, there was no correlation between GH tumor subtype and IGF-1 levels [62]. The short-term follow up in this study limits ability to predict long-term response to SSA therapy.

Recently, Bhayana and coworkers performed a 10-year retrospective analysis to assess the correlation of GH tumor type and response to SSA therapy [23]. All 40 patients in this cohort underwent transsphenoidal surgery followed by octreotide administration for a minimum of 4 months. Complete response to therapy was defined as normalization of age-adjusted IGF-1; and corroborated by a random GH $<$1.0 μg/L or suppressed GH $<$ 1.0 μg/L following 75-g oral glucose tolerance test (OGTT). Responders (20 patients) to SSA therapy included 80 % of patients with DG tumors compared with 20 % of those with SG tumors; 70 % (16/23) of all DG and 31 % (4/13) all SG were responders. The authors concluded that tumor morphology is an important predictor of response to medical therapy with SSAs [23]. The non-responder group (19 patients) in this study was composed of 44 % DG and 56 % SG tumors, again highlighting the fact that additional factors, beyond tumor morphology likely play an important role in response to medical therapy. Correlation of GH tumor subtypes and response to pegvisomant, GH receptor antagonist, has not been reported to date.

Conclusion

Growth hormone–secreting adenomas, the major cause of acromegaly in adults, are associated with significant morbidity as well as decreased lifespan when disease remains active or reoccurs [2]. Identifying potentially aggressive GH tumors early would not only have a prognostic value but would also guide the intensity of therapy. In the past, studies have suggested that the clinical predictors of more aggressive GH tumors included younger age, larger invasive tumors and higher basal GH levels and IGF-1 levels. In the last two decades, studies have examined histologic subtypes of GH pituitary tumors in correlation to tumor behavior and treatment response. Although most studies imply that SG adenomas are more commonly associated with aggressive features such as local invasion, suprasellar extension and cavernous sinus invasion, histological subtyping into SG versus DG still does not absolutely predict tumor behavior [31]. Molecular mechanisms underlying the morphologically different GH tumors subtypes are only partially understood and cannot explain the variable behavior of these two subtypes. Elucidating differential mechanisms and pathways between DG and SG tumors and new molecular biomarkers may lead to better disease prognostication as well as potentially superior therapeutic targets. A further advantage of future molecular studies would be the identification of markers that could be adapted for immunohistochemical evaluation and diagnosis of GH adenomas in routine diagnostic practice.

Conflict of interest The authors report no conflicts of interest concerning the material and findings specified in this article.

References

1. S.L. Asa, S. Ezzat, The pathogenesis of pituitary tumors. Annu. Rev. Pathol. **4**, 97–126 (2009). doi:10.1146/annurev.pathol.4.110807.092259
2. S. Melmed, Acromegaly pathogenesis and treatment. J. Clin. Invest. **119**(11), 3189–3202 (2009). doi:10.1172/JCI3937539375
3. I. Shimon, Z.R. Cohen, Z. Ram, M. Hadani, Transsphenoidal surgery for acromegaly: endocrinological follow-up of 98 patients. Neurosurgery **48**(6), 1239–1243 (2001). Discussion 1244–1235
4. A. Giustina, P. Chanson, M.D. Bronstein, A. Klibanski, S. Lamberts, F.F. Casanueva, P. Trainer, E. Ghigo, K. Ho, S. Melmed, A consensus on criteria for cure of acromegaly. J. Clin. Endocrinol. Metab. **95**(7), 3141–3148 (2010). doi:10.1210/jc.2009-2670
5. S. Melmed, A. Colao, A. Barkan, M. Molitch, A.B. Grossman, D. Kleinberg, D. Clemmons, P. Chanson, E. Laws, J. Schlechte, M.L. Vance, K. Ho, A. Giustina, Guidelines for acromegaly management: an update. J. Clin. Endocrinol. Metab. **94**(5), 1509–1517 (2009). doi:10.1210/jc.2008-2421
6. I. Donangelo, S. Melmed, Treatment of acromegaly: future. Endocrine **28**(1), 123–128 (2005). doi:10.1385/ENDO:28:1:123
7. A. Stevenaert, A. Beckers, Presurgical Octreotide: treatment in acromegaly. Metabolism **45**(8 Suppl 1), 72–74 (1996)
8. D.R. Clemmons, K. Chihara, P.U. Freda, K.K. Ho, A. Klibanski, S. Melmed, S.M. Shalet, C.J. Strasburger, P.J. Trainer, M.O. Thorner, Optimizing control of acromegaly: integrating a growth hormone receptor antagonist into the treatment algorithm. J. Clin. Endocrinol. Metab. **88**(10), 4759–4767 (2003)
9. J.D. Carmichael, V.S. Bonert, J.M. Mirocha, S. Melmed, The utility of oral glucose tolerance testing for diagnosis and assessment of treatment outcomes in 166 patients with acromegaly. J. Clin. Endocrinol. Metab. **94**(2), 523–527 (2009). doi:10.1210/jc.2008-1371

10. A. Giustina, T. Porcelli, Pituitary gland: medical therapy for acromegaly: can we predict response? Nat. Rev. Endocrinol. **5**(8), 425–427 (2009). doi:10.1038/nrendo.2009.146
11. P.U. Freda, Somatostatin analogs in acromegaly. J. Clin. Endocrinol. Metab. **87**(7), 3013–3018 (2002)
12. P.J. Trainer, S. Ezzat, G.A. D'Souza, G. Layton, C.J. Strasburger, A randomized, controlled, multicentre trial comparing pegvisomant alone with combination therapy of pegvisomant and long-acting octreotide in patients with acromegaly. Clin. Endocrinol. **71**(4), 549–557 (2009). doi:10.1111/j.1365-2265.2009.03620.x
13. P. Lundin, B. Eden Engstrom, F.A. Karlsson, P. Burman, Long-term octreotide therapy in growth hormone-secreting pituitary adenomas: evaluation with serial MR. AJNR Am. J. Neuroradiol. **18**(4), 765–772 (1997)
14. C. Salaun, L. Foubert, M. Vialatou, M. Kujas, G. Turpin, Prognostic factors in the surgical management of acromegaly. Ann. Med. Interne **150**(3), 195–198 (1999)
15. P. Nomikos, M. Buchfelder, R. Fahlbusch, The outcome of surgery in 668 patients with acromegaly using current criteria of biochemical 'cure'. Eur. J. Endocrinol. **152**(3), 379–387 (2005). doi:10.1530/eje.1.01863
16. A. Abosch, J.B. Tyrrell, K.R. Lamborn, L.T. Hannegan, C.B. Applebury, C.B. Wilson, Transsphenoidal microsurgery for growth hormone-secreting pituitary adenomas: initial outcome and long-term results. J. Clin. Endocrinol. Metab. **83**(10), 3411–3418 (1998)
17. Roelfsema, F., Biermasz, N.R., Pereira, A.M.: Clinical factors involved in the recurrence of pituitary adenomas after surgical remission: a structured review and meta-analysis. Pituitary (2011). doi:10.1007/s11102-011-0347-7
18. A. Fusco, M.C. Zatelli, A. Bianchi, V. Cimino, L. Tilaro, F. Veltri, F. Angelini, L. Lauriola, V. Vellone, F. Doglietto, M.R. Ambrosio, G. Maira, A. Giustina, E.C. degli Uberti, A. Pontecorvi, L. De Marinis, Prognostic significance of the Ki-67 labeling index in growth hormone-secreting pituitary adenomas. J. Clin. Endocrinol. Metab. **93**(7), 2746–2750 (2008). doi:10.1210/jc.2008-0126
19. C.H. Botelho, A.V. Magalhaes, P.A. Mello, F.C. Schmitt, L.A. Casulari, Expression of p53, Ki-67 and c-erb B2 in growth hormone-and/or prolactin-secreting pituitary adenomas. Arq. Neuropsiquiatr. **64**(1), 60–66 (2006). doi:/S0004-282X2006000100013
20. K. Yonezawa, N. Tamaki, T. Kokunai, Clinical features and growth fractions of pituitary adenomas. Surg. Neurol. **48**(5), 494–500 (1997)
21. S.S. Zuhur, C. Tanik, O. Karaman, S. Velet, E. Cil, F.Y. Ozturk, H. Ozkayalar, A.M. Musluman, Y. Altuntas, MGMT immunoexpression in growth hormone-secreting pituitary adenomas and its correlation with Ki-67 labeling index and cytokeratin distribution pattern. Endocrine **40**(2), 222–227 (2011). doi:10.1007/s12020-011-9485-y
22. G. Kontogeorgos, Classification and pathology of pituitary tumors. Endocrine **28**(1), 27–35 (2005). doi:10.1385/ENDO:28:1:027
23. S. Bhayana, G.L. Booth, S.L. Asa, K. Kovacs, S. Ezzat, The implication of somatotroph adenoma phenotype to somatostatin analog responsiveness in acromegaly. J. Clin. Endocrinol. Metab. **90**(11), 6290–6295 (2005). doi:10.1210/jc.2005-0998
24. Asa, S.L.: Tumors of the pituitary gland. Atlas of Tumor Pathology, vol. Third Series; Fasicle 22. Armed Forces Institute of Pathology, Washington, DC (1998)
25. M.B. Lopes, Growth hormone-secreting adenomas: pathology and cell biology. Neurosurg. Focus **29**(4), E2 (2010). doi:10.3171/2010.7.FOCUS10169
26. K. Kovacs, E. Horvath, B. Corenblum, A.M. Sirek, G. Penz, C. Ezrin, Pituitary chromophobe adenomas consisting of prolactin cells: a histologic, immunocytological and electron microscopic study. Virchows Arch. A Pathol. Anat. Histol. **366**(2), 113–123 (1975)
27. E. Horvath, K. Kovacs, Ultrastructural classification of pituitary adenomas. Can. J. Neurol. Sci. **3**(1), 9–21 (1976)
28. T. Sano, T. Ohshima, S. Yamada, Expression of glycoprotein hormones and intracytoplasmic distribution of cytokeratin in growth hormone-producing pituitary adenomas. Pathol. Res. Pract. **187**(5), 530–533 (1991)
29. V. Karantza, Keratins in health and cancer: more than mere epithelial cell markers. Oncogene **30**(2), 127–138 (2011). doi:10.1038/onc.2010.456
30. H. Bando, T. Sano, T. Ohshima, C.Y. Zhang, R. Yamasaki, K. Matsumoto, S. Saito, Differences in pathological findings and growth hormone responses in patients with growth hormone-producing pituitary adenoma. Endocrinol. Jpn. **39**(4), 355–363 (1992)
31. A. Obari, T. Sano, K. Ohyama, E. Kudo, Z.R. Qian, A. Yoneda, N. Rayhan, M. Mustafizur Rahman, S. Yamada, Clinicopathological features of growth hormone-producing pituitary adenomas: difference among various types defined by cytokeratin distribution pattern including a transitional form. Endocr. Pathol. **19**(2), 82–91 (2008). doi:10.1007/s12022-008-9029-z
32. P.R. Mazal, T. Czech, R. Sedivy, M. Aichholzer, J. Wanschitz, N. Klupp, H. Budka, Prognostic relevance of intracytoplasmic cytokeratin pattern, hormone expression profile, and cell proliferation in pituitary adenomas of akromegalic patients. Clin. Neuropathol. **20**(4), 163–171 (2001)
33. S. Yamada, T. Aiba, T. Sano, K. Kovacs, Y. Shishiba, S. Sawano, K. Takada, Growth hormone-producing pituitary adenomas: correlations between clinical characteristics and morphology. Neurosurgery **33**(1), 20–27 (1993)
34. Y. Bakhtiar, H. Hirano, K. Arita, S. Yunoue, S. Fujio, A. Tominaga, T. Sakoguchi, K. Sugiyama, K. Kurisu, J. Yasufuku-Takano, K. Takano, Relationship between cytokeratin staining patterns and clinico-pathological features in somatotropinomae. Eur. J. Endocrinol. **163**(4), 531–539 (2010). doi:10.1530/EJE-10-0586
35. J. Kreutzer, M.L. Vance, M.B. Lopes, E.R. Laws Jr, Surgical management of GH-secreting pituitary adenomas: an outcome study using modern remission criteria. J. Clin. Endocrinol. Metab. **86**(9), 4072–4077 (2001)
36. I.A. Felix, E. Horvath, K. Kovacs, H.S. Smyth, D.W. Killinger, J. Vale, Mammosomatotroph adenoma of the pituitary associated with gigantism and hyperprolactinemia. A morphological study including immunoelectron microscopy. Acta Neuropathol. **71**(1–2), 76–82 (1986)
37. S.L. Fougner, O. Casar-Borota, A. Heck, J.P. Berg, J. Bollerslev, Adenoma granulation pattern correlates with clinical variables and effect of somatostatin analogue treatment in a large series of patients with acromegaly. Clin. Endocrinol. **76**(1), 96–102 (2012). doi:10.1111/j.1365-2265.2011.04163.x
38. J. Hardy, Transsphenoidial microsurgical treatment of pituitary tumours, in *Recent Advances in the Diagnosis and Treatment of Pituitary Tumours*, ed. by L. Linfoot (Raven Press, New York, 1979), pp. 375–387
39. E. Knosp, E. Steiner, K. Kitz, C. Matula, Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery **33**(4), 610–617 (1993). Discussion: 617–618
40. A. Hagiwara, Y. Inoue, K. Wakasa, T. Haba, T. Tashiro, T. Miyamoto, Comparison of growth hormone-producing and non-growth hormone-producing pituitary adenomas: imaging characteristics and pathologic correlation. Radiology **228**(2), 533–538 (2003). doi:10.1148/radiol.2282020695228202069 5
41. A. Heck, G. Ringstad, S.L. Fougner, O. Casar-Borota, T. Nome, J. Ramm-Pettersen, J. Bollerslev, Intensity of pituitary adenoma

on T2 weighted MRI predicts the response to octreotide treatment in newly diagnosed acromegaly. Clin. Endocrinol. (2011). doi:10.1111/j.1365-2265.2011.04286.x
42. S. Ahmed, M. Elsheikh, I.M. Stratton, R.C. Page, C.B. Adams, J.A. Wass, Outcome of transphenoidal surgery for acromegaly and its relationship to surgical experience. Clin. Endocrinol. **50**(5), 561–567 (1999)
43. C. Beauregard, U. Truong, J. Hardy, O. Serri, Long-term outcome and mortality after transsphenoidal adenomectomy for acromegaly. Clin. Endocrinol. **58**(1), 86–91 (2003)
44. M. Arosio, M.A. Giovanelli, E. Riva, C. Nava, B. Ambrosi, G. Faglia, Clinical use of pre- and postsurgical evaluation of abnormal GH responses in acromegaly. J. Neurosurg. **59**(3), 402–408 (1983). doi:10.3171/jns.1983.59.3.0402
45. S.J. Brockmeier, M. Buchfelder, R. Fahlbusch, TRH/GnRH test in acromegaly. Long-term follow-up experience with successfully treated patients. Horm. Metab. Res. **25**(5), 275–277 (1993). doi:10.1055/s-2007-1002096
46. N.R. Biermasz, J.W. Smit, H. van Dulken, F. Roelfsema, Postoperative persistent thyrotrophin releasing hormone-induced growth hormone release predicts recurrence in patients with acromegaly. Clin. Endocrinol. **56**(3), 313–319 (2002)
47. S. Valdemarsson, S. Ljunggren, M. Bramnert, O. Norrhamn, C.H. Nordstrom, Early postoperative growth hormone levels: high predictive value for long-term outcome after surgery for acromegaly. J. Intern. Med. **247**(6), 640–650 (2000)
48. C.L. Ronchi, V. Varca, C. Giavoli, P. Epaminonda, P. Beck-Peccoz, A. Spada, M. Arosio, Long-term evaluation of postoperative acromegalic patients in remission with previous and newly proposed criteria. J. Clin. Endocrinol. Metab. **90**(3), 1377–1382 (2005). doi:10.1210/jc.2004-1974
49. L. De Marinis, A. Mancini, A. Bianchi, R. Gentilella, D. Valle, A. Giampietro, P. Zuppi, C. Anile, G. Maira, A. Giustina, Preoperative growth hormone response to thyrotropin-releasing hormone and oral glucose tolerance test in acromegaly: a retrospective evaluation of 50 patients. Metab. Clin. Exp. **51**(5), 616–621 (2002)
50. B. Xu, T. Sano, K. Yoshimoto, S. Yamada, Downregulation of E-cadherin and its undercoat proteins in pituitary growth hormone cell adenomas with prominent fibrous bodies. Endocr. Pathol. **13**(4), 341–351 (2002). doi:EP:13:4:341
51. T. Sano, Q.Z. Rong, N. Kagawa, S. Yamada, Down-regulation of E-cadherin and catenins in human pituitary growth hormone-producing adenomas. Front. Horm. Res. **32**, 127–132 (2004)
52. H. McNeill, M. Ozawa, R. Kemler, W.J. Nelson, Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. Cell **62**(2), 309–316 (1990). doi:0092-8674(90)90368-O
53. T. Brabletz, F. Hlubek, S. Spaderna, O. Schmalhofer, E. Hiendlmeyer, A. Jung, T. Kirchner, Invasion and metastasis in colorectal cancer: epithelial–mesenchymal transition, mesenchymal–epithelial transition, stem cells and beta-catenin. Cells Tissues Organs **179**(1–2), 56–65 (2005). doi:10.1159/000084509
54. M.J. Wheelock, K.R. Johnson, Cadherins as modulators of cellular phenotype. Annu. Rev. Cell Dev. Biol. **19**, 207–235 (2003). doi:10.1146/annurev.cellbio.19.011102.111135
55. L. Vallar, A. Spada, G. Giannattasio, Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. Nature **330**(6148), 566–568 (1987). doi:10.1038/330566a0
56. C.A. Landis, S.B. Masters, A. Spada, A.M. Pace, H.R. Bourne, L. Vallar, GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature **340**(6236), 692–696 (1989). doi:10.1038/340692a0
57. A. Spada, M. Arosio, D. Bochicchio, N. Bazzoni, L. Vallar, M. Bassetti, G. Faglia, Clinical, biochemical, and morphological correlates in patients bearing growth hormone-secreting pituitary tumors with or without constitutively active adenylyl cyclase. J. Clin. Endocrinol. Metab. **71**(6), 1421–1426 (1990)
58. F.H. Burton, K.W. Hasel, F.E. Bloom, J.G. Sutcliffe, Pituitary hyperplasia and gigantism in mice caused by a cholera toxin transgene. Nature **350**(6313), 74–77 (1991). doi:10.1038/350074a0
59. S.L. Asa, R. Digiovanni, J. Jiang, M.L. Ward, K. Loesch, S. Yamada, T. Sano, K. Yoshimoto, S.J. Frank, S. Ezzat, A growth hormone receptor mutation impairs growth hormone autofeedback signaling in pituitary tumors. Cancer Res. **67**(15), 7505–7511 (2007). doi:10.1158/0008-5472.CAN-07-0219
60. B. Kola, M. Korbonits, S. Diaz-Cano, G. Kaltsas, D.G. Morris, S. Jordan, L. Metherell, M. Powell, S. Czirjak, G. Arnaldi, S. Bustin, M. Boscaro, F. Mantero, A.B. Grossman, Reduced expression of the growth hormone and type 1 insulin-like growth factor receptors in human somatotroph tumours and an analysis of possible mutations of the growth hormone receptor. Clin. Endocrinol. **59**(3), 328–338 (2003)
61. S.L. Asa, K.T. Coschigano, L. Bellush, J.J. Kopchick, S. Ezzat, Evidence for growth hormone (GH) autoregulation in pituitary somatotrophs in GH antagonist-transgenic mice and GH receptor-deficient mice. Am. J. Pathol. **156**(3), 1009–1015 (2000). doi:10.1016/S0002-9440(10)64968-1
62. S. Ezzat, G. Kontogeorgos, D.A. Redelmeier, E. Horvath, A.G. Harris, K. Kovacs, In vivo responsiveness of morphological variants of growth hormone-producing pituitary adenomas to octreotide. Eur. J. Endocrinol. **133**(6), 686–690 (1995)