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Pathology of the Adrenal Cortex: a Reappraisal of the Past 25 Years Focusing on Adrenal Cortical Tumors

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Abstract A reappraisal of the major advances in the diagnostic pathology of adrenal cortical lesions and tumors in the last 25 years is presented, with special reference to the definition of malignancy in primary adrenal cancer and its variants. Slightly more than 25 years ago, Weiss proposed his diagnostic scoring system for adrenal cortical carcinoma. This represented a milestone for adrenal pathologists and the starting point for further modifications of the system, either through minor changes in the scoring procedure itself or concentrating on some particular Weiss criterion such as mitotic index, integrated into alternative scoring schemes or algorithms that are currently under validation. Improvements in diagnostic immunohistochemistry have led to the identification of markers of cortical origin, such as Melan-A, alpha-inhibin, and SF-1 and of prognostic factors in carcinoma, such as the Ki-67 proliferation index and SF-1 itself. With regard to hyperplastic conditions, genetic investigations have allowed the association of the majority of cases of primary pigmented nodular adrenocortical disease (PPNAD) in Carney complex to mutations in the gene encoding the regulatory subunit 1A of protein kinase A (*PRKAR1A*). Other hereditary conditions are also associated with adrenal cortical tumors, including the Li–Fraumeni, Beckwith–Wiedemann, Gardner, multiple endocrine neoplasia type 1, and neurofibromatosis type 1 syndromes. Moreover, several advances have been made in the knowledge of the molecular background of sporadic tumors, and a number of molecules/genes are of particular interest as potential diagnostic and prognostic biomarkers.

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Introduction: Where We Were

Diagnostic pathology of adrenal cortical diseases classically included diffuse lesions and single or multiple nodular conditions. In this respect, the diagnostic approach has not undergone major changes in the past 25 years, but several new tools have become available for the purpose of better identifying different diseases and tumor categories. Light microscopy and immunophenotyping or molecular markers have led to the definition of more accurate and reproducible categories of adrenal cortical disorders, especially in neoplastic diseases.

Twenty-five years ago, pathology reports were based on few terms (mainly hyperplasia, adenoma, carcinoma, or metastasis), and a description of the relevant pathological features was attached, according to the local practice of different countries and institutions. Electron microscopy was generally not necessary for subtyping adrenal cortical diseases, immunohistochemical diagnostic markers related to adrenal cortex were very limited, and no prognostic or predictive markers were known. However, as for other types of human diseases, pathology of the adrenal cortex has progressed—especially in the most recent years—together with the improvement of the clinical diagnosis and management of patients, and with the technical implementation of ancillary tools.

This overview will summarize the major advances in the diagnosis of adrenal cortical lesions, separately approaching diagnostic, immunophenotypic, and molecular data that have emerged in the past 25 years, focusing on adrenal cortical tumors and on novel insights that have improved the diagnostic workup or are currently being validated. A summary of the most relevant achievements is illustrated in Fig. 1.

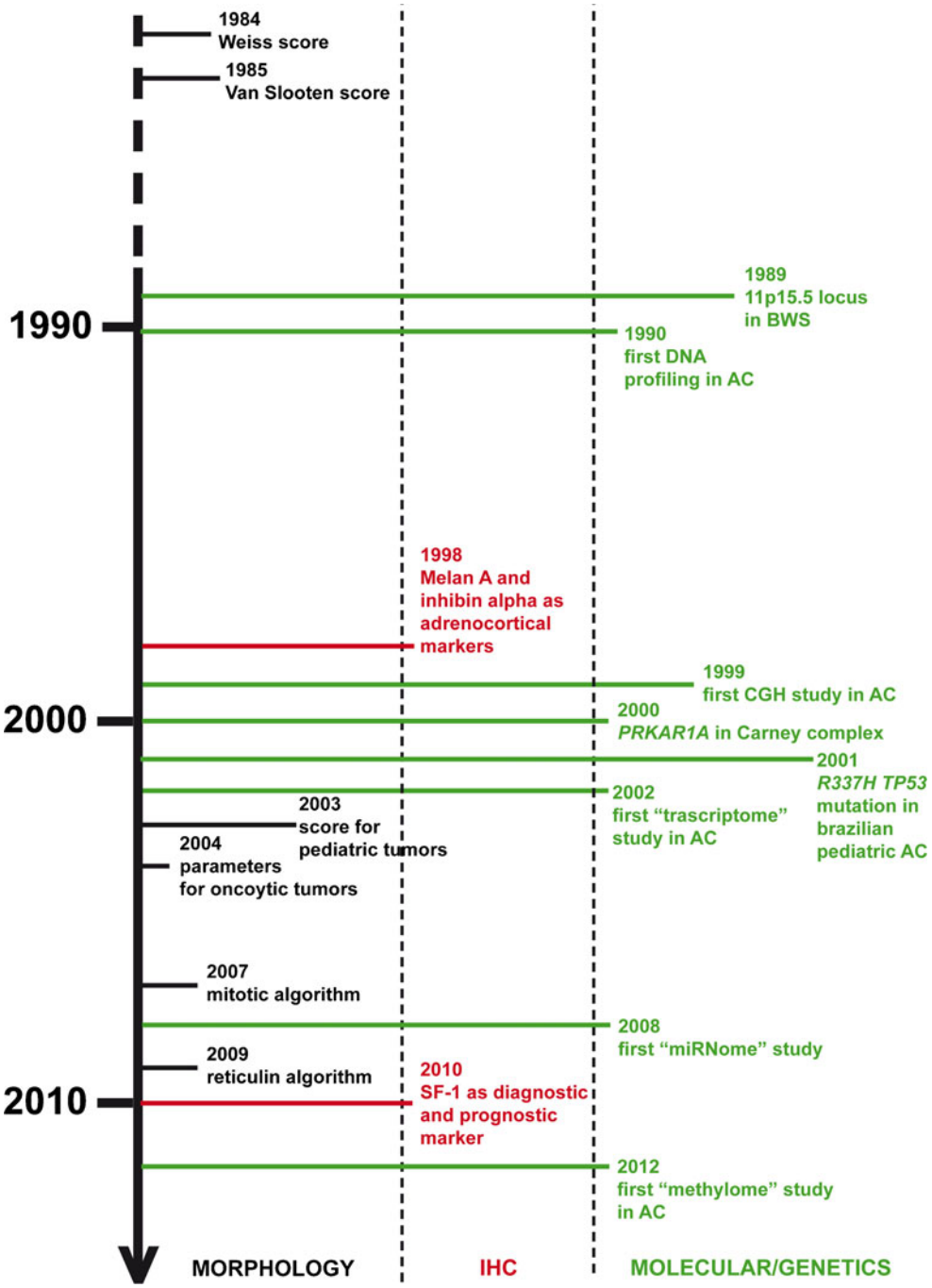

Fig. 1 Milestones in the pathological and molecular characterization of adrenal diseases in the past 25 years

Diagnostic Criteria of Malignancy

Twenty-five years ago several parameters of malignancy had been identified, all having a poor sensitivity for adrenal cortical carcinoma diagnosis. As a result, Weiss proposed combining some of them into a scoring system. This proposal (the Weiss score) was a major advance in adrenal cortical cancer pathology and still represents—in its original proposal [1, 2] or after minor modifications [3]—a milestone in this field. However, the effects of a 25-year-long application of the Weiss score, as also discussed by Weiss himself in a recent reappraisal of his score [4], led to the recognition that the relevance and reproducibility of the individual parameters vary.

As a direct consequence of this situation, alternative diagnostic protocols or algorithms have emerged in the last decades, all having a limited application and a lower popularity compared to the Weiss score, at least until recent years. As a matter of fact, all systems used for adrenal cortical carcinoma diagnosis largely rely on mitotic figures count, which thus

represents the most relevant and accurate parameter to assess malignancy in adrenal cortical tumors, irrespective of the diagnostic protocol applied. In this respect, Diaz-Cano and Blanes proposed a diagnostic algorithm based on the mitotic count and suggested that a cutoff value of >5 mitotic figures in 50 high power fields was associated per se with malignant behavior [5]. Among other alternative approaches, an algorithm was recently designed to combine architectural features (namely the disruption of the reticulin framework) and the presence of malignancy-related parameters already included in the Weiss score, such as increased mitotic index and the presence of necrosis and vascular invasion [6] (Fig. 2). This algorithm was able to reclassify with a 100 % sensitivity and specificity all cases with a "malignant" Weiss score (>3) and is generally simple and easier to apply. In addition, in subsequent publications, it proved applicable also in specific adrenal cortical tumor variants, namely the myxoid and the oncocytic subtypes (see also below) [7, 8].

On the other hand, it soon became clear that some parameters proposed in the Weiss score (including sinusoidal invasion, diffuse growth, etc.) were difficult to apply and lacked interobserver reproducibility. These difficulties were eventually addressed in a recent study reported by a French group [9], which definitely demonstrated that not all Weiss criteria are easily applicable and that pathologists' training is crucial in improving the diagnostic accuracy. Moreover, in a recent study, the reproducibility of reticulin staining—which is the key parameter of the algorithm described above—was also tested among observers of different centers that blindly examined over 240 adrenal cortical tumors; the assessment of reticulin disruption was highly reproducible among pathologists, especially after specific training and in cases classified as malignant according to the Weiss score [10].

Special Types of Adrenal Cortical Tumors

Oncocytic Adrenal Cortical Tumors

Oncocytic adrenal cortical neoplasms represent a subset of tumors with a predominant component of usually large cells with the cytoplasm filled with mitochondria which confer a granular and deeply eosinophilic appearance. Since the first report in 1986 [11], many case reports or small series have been published. In the series collected at our institution, the prevalence of oncocytic adrenal cortical carcinomas among malignant tumors was approximately 18 % [8]. Oncocytic adrenal cortical neoplasms may be classified into "pure" if made of >90 % oncocytes, mixed if oncocytes are present in 50 to 90 % of the tumor, and focal if the oncocytic component accounts for less than 50 %. As originally stated by Bisceglia et al. [12], the diagnosis of malignancy in oncocytic tumors is difficult using the classical Weiss score, since at least three parameters (eosinophilic cytoplasm, high nuclear grade, and diffuse architecture) are intrinsically present in this tumor type, irrespective of the biological and clinical behavior. As a consequence, the cutoff values validated for conventional adrenal cortical carcinoma may lead to an overdiagnosis of malignancy in this special group. For this reason, exclusively for purely oncocytic tumors, an alternative diagnostic system was proposed based on criteria specific for this tumor type: any of the three "major criteria" (mitotic rate >5 per 50 HPF, atypical mitoses, and venous invasion) defines an oncocytic adrenal cortical carcinoma, whereas one to four "minor criteria" (necrosis, capsular and sinusoidal invasion, size >10 cm, or weight >200 g) define an oncocytic adrenal cortical neoplasm of borderline malignancy [12]. A retrospective survival analysis provided by Wong and coworkers [13] showed that this classification correctly predicted the malignant potential of these tumors and demonstrated a generally better prognosis for oncocytic carcinomas in comparison with those of the classical type. A further study claimed that the reticulin algorithm correctly stratifies oncocytic adrenal cortical tumors into benign and malignant and confirmed the "low-grade" malignancy for these tumors, as also supported by low mean mitotic and Ki-67 indexes [8]. Moreover, as for oncocytic neoplasms at other sites [14, 15], the mitochondrial DNA "common deletion" (4,977 base pairs) was identified in approximately 40 % of oncocytic adrenal cortical tumors, more commonly in borderline or benign tumors, but not in control adrenal cortical carcinomas of the classical type [8].

Myxoid Adrenal Cortical Tumors

The description of myxoid adrenal cortical tumors dates back to 1979 [16], and these neoplasms are characterized by extracellular deposits of myxoid material, highlighted by positive Alcian Blue staining, which can be predominant in a subset of approximately 10 % of ACC [7, 17]. Among them, two groups can be identified based on architectural and cytological features, irrespective of the amount of myxoid material: group 1 tumors are characterized by monotonous cells of small to medium size, with mild to moderate nuclear atypia and scant, lightly eosinophilic cytoplasm, growing in a trabecular, pseudoglandular, or cribriform pattern; conversely, group 2 tumors are more similar to classical adrenal cortical carcinomas, comprising large pleomorphic cells with a moderate to high nuclear atypia and abundant eosinophilic cytoplasm, growing in a diffuse pattern and usually having focal myxoid areas (<20 % of the total area) within an otherwise conventional adrenal cancer. Although some cases show a certain degree of overlapping features between these two groups, group 2 tumors are probably the result of degenerative myxoid changes in conventional adrenal cortical carcinomas, and we suggest that they should not be classified into this distinctive variant of adrenal cortical tumors.

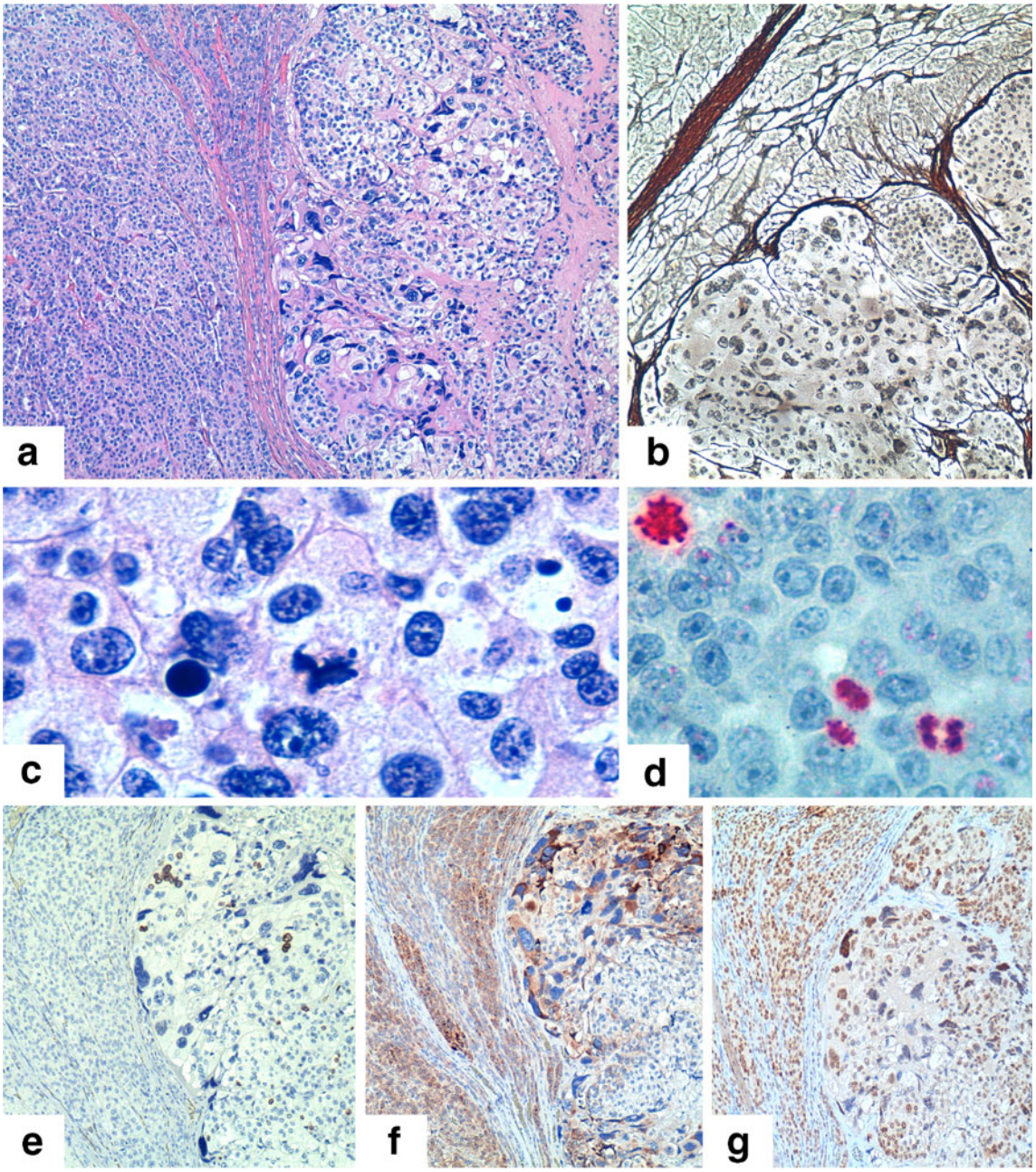

Fig. 2 Pathological characteristics of a case of adrenal carcinoma (2.5 cm in the largest dimension) showing residual peripheral "adenomatous" features (*left part* in **a**, **e**, **f**, **g**; *upper left corner* in **b**). The carcinoma component showed reticulin disruption (**b**), atypical mitotic figures (**c**), increased mitotic index (as highlighted by phospho-histone H3, **d**), and increased Ki-67 index (**e**); adrenocortical markers such as Melan-A (**f**) and SF-1 (**g**) were positive in both components (original magnifications: ×100 in all figures except **c** and **d**, ×400; **a**, **c** H&E; **b** silver staining; **d** immunoperoxidase with AEC as chromogen; **e**, **f**, **g** immunoperoxidase with diaminobenzidine as chromogen)

Based on the aforementioned pathological features, the myxoid variant is challenging for several reasons. The first is the differential diagnosis, especially in biopsy material, with other myxoid neoplasms, such as extra-skeletal myxoid chondrosarcoma, chordoma, myxoid carcinomas from the kidney or other sites, and myxoid lipomatous or nerve sheath tumors. Secondly, the distinction between benign and malignant adrenal cortical myxoid tumors may be difficult. Due to the abovementioned cyto-architectural characteristics, myxoid tumors often lack two of the Weiss parameters (diffuse growth and nuclear atypia), and the identification of invasive areas might be hard or equivocal in the presence of abundant myxoid background. Therefore, in adrenal tumors with predominant myxoid stroma, it is advisable to consider malignancy even in tumors with a low-grade appearance. Moreover, in terms of prognosis, it has also been suggested that myxoid adrenal cortical carcinomas may have a more aggressive clinical behavior [17].

Sarcomatoid Variant of Adrenal Cortical Carcinoma

This is the rarest adrenal cortical carcinoma variant, and to our knowledge, only 14 cases have been reported in the literature so far. They have been classified as either sarcomatoid carcinomas with spindle cell areas [18–22] or carcinosarcomas in the presence of a specialized mesenchymal component, revealing osteosarcomatous [23, 24], chondrosarcomatous [23], rhabdomyosarcomatous [25–28], and primitive neuroectodermal tumor-like [28] differentiation. Sarcomatoid carcinoma is highly aggressive, and the recognition of diagnostic features of malignancy is not problematic. For this reason, a cutoff of 10 % of sarcomatous component to diagnose this unusual variant, irrespective of the Weiss score [21], was suggested.

Pediatric Adrenal Cortical Tumors

Pediatric adrenal cortical tumors are rarer than their adult counterparts, with an incidence which varies across geographic regions, being remarkably high in Southern Brazil, compared to the USA and Europe. The diagnosis of malignancy in pediatric adrenal cortical tumors is even more challenging than those of adults, because the Weiss score criteria often lack sensitivity and specificity in pediatric cases. For this reason, in 2003, Wieneke and coworkers [29] proposed categorizing pediatric adrenal cortical tumors into three different prognostic groups, "benign," "indeterminate for malignancy," and "malignant," according to the evaluation of nine parameters that were found the most statistically significant to predict malignant behavior. In this study, among the classical criteria considered in the Weiss system, only confluent necrosis, capsular or vascular invasion, presence of atypical mitotic figures, and a mitotic count >15 per 20 high power fields were significantly related to poor prognosis. Conversely, some others (eosinophilic cytoplasm, diffuse architecture, or sinusoidal invasion) were apparently less relevant. Four additional parameters had also a significant impact on prognosis, including tumor weight >400 g, tumor size >10.5 cm, vena cava invasion, and peri-adrenal tissue infiltration. The prognostic relevance of these parameters has recently been validated in the Italian Pediatric Rare Tumor (TREP) Study [30]. Moreover, in this latter series, the authors found that focal myxoid stromal changes, which were not included in the Wieneke system, were also suggestive of malignancy in pediatric tumors.

Mixed Cortico-medullary Tumors

Mixed cortico-medullary adrenal tumors are rare, with fewer than 20 well-documented cases of mixed cortico-medullary adenomas. Most cases are benign and show a dual divergent differentiation, as confirmed by the concurrent expression of adrenal cortex and adrenal medulla markers. Moreover, a case showing mixed cortical and medullary histological characteristics, as well as gross and microscopic evidence of malignancy, has recently been documented [31].

Immunophenotypic Markers

In adrenal cortical tumor pathology, immunohistochemistry has chronologically become useful for the purpose of differential diagnosis, firstly between adrenal cortical carcinoma and extra-adrenal neoplasms (renal cell carcinoma, melanoma, poorly differentiated metastatic carcinomas, retroperitoneal sarcomas) (Fig. 3), then between adrenal cortical and other primary adrenal tumors (pheochromocytoma, PEComa), and more recently between adrenal cortical adenoma and carcinoma. In addition, some immunohistochemical markers have been proposed as prognostic tools, although few of them have so far been validated in independent cohorts.

Diagnostic Markers of Adrenal Cortical Origin

Many malignant neoplasms metastasize to the adrenal gland including lung, gastrointestinal, renal and breast cancer, and melanoma [32], and reliable immunohistochemical markers are therefore required to establish the correct diagnosis. Adrenal cortical tumors are usually positive for a variety of markers that are common to several other malignancies, such as vimentin [33], and variably express cytokeratin and neuroendocrine markers, such as synaptophysin and neurofilament. Moreover, adrenal cortex as well as adrenocortical tumors nearly invariably express mesothelioma-related markers calretinin [34] and D2-40 [35], although the specificity for the adrenocortical origin of these markers is limited by their expression at a variable extent also in other neoplasms including abdominal ones (i.e., renal cell carcinoma, ovarian carcinoma).

In 1990, a monoclonal antibody (D11) highly specific for adrenal cortical tissue was described [36, 37]. However, its immunoreactivity was later observed only in a small subset of adrenal cortical carcinomas, heavily restricting its use as a general marker for adrenal cortical derivation [38, 39]. Moreover, D11 is no longer commercially available or otherwise accessible.

Since 1998, Melan-A (MART-1) and alpha-inhibin have become the most commonly used markers to prove the adrenal cortical origin of a given lesion [40–44]. Alpha-inhibin was also demonstrated to reflect the hormonal secretion of the tumor, being more frequently and intensively expressed in androgen-secreting neoplasms [45]. However, both markers have a limited sensitivity and fail to recognize a significant proportion (28 % for Melan-A and 31 % for alpha-inhibin) of adrenal cortical carcinomas.

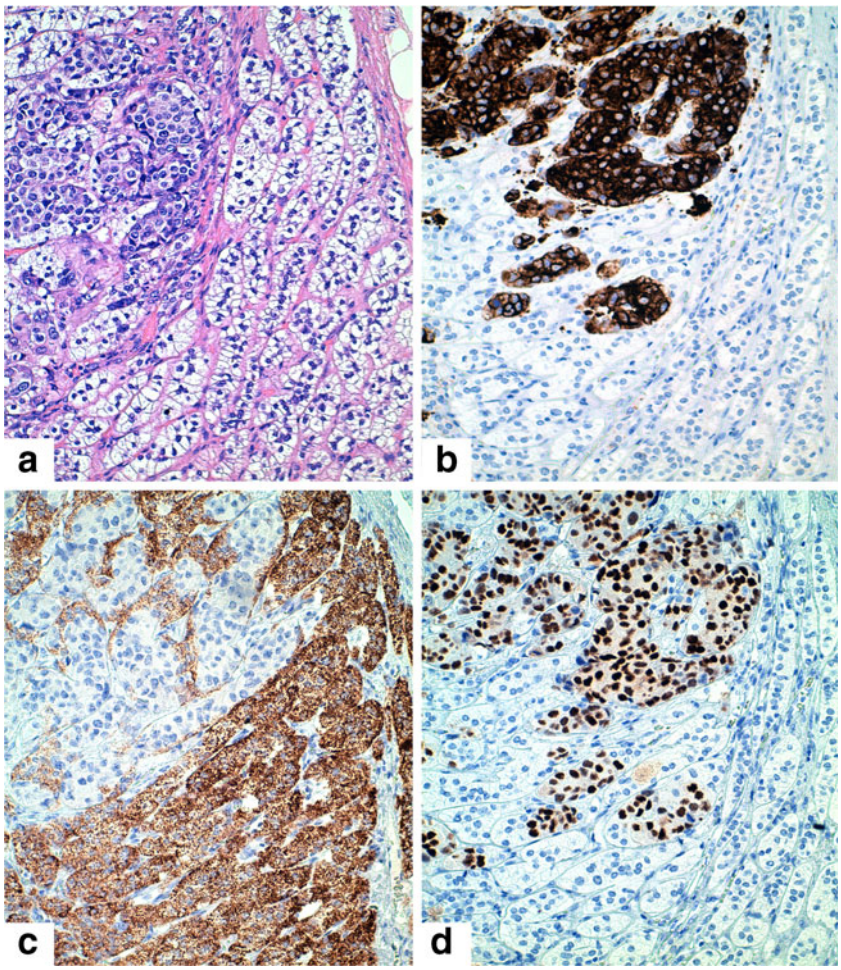

Fig. 3 Morphological and immunophenotypical profile of a breast carcinoma metastatic into an adrenal adenoma. Breast cancer cells (**a** to **d**, *upper left corner*) were immunoreactive for pan-cytokeratins (**b**) and estrogen receptors (**d**) but negative for Melan-A (**c**) (original magnifications: ×200 in all figures; **a** H&E; **b**–**d** immunoperoxidase with diaminobenzidine as chromogen)

Although already described in 1995 by Sasano and coworkers [46], only recently has steroidogenic factor-1 (SF-1; also known as Ad4BP or NR5A1) progressively become the marker of choice to differentiate between tumors of adrenal cortical and nonadrenal cortical origin. Physiologically, it plays a key role in the development of steroidogenic tissues and in the regulation of steroid biosynthesis and was found to be overexpressed in most cases of childhood adrenal cortical tumors [47, 48]. In 2010, Sbiera and coworkers [49] demonstrated in a very large series of cases that nuclear SF-1 staining is a highly sensitive and specific marker of adrenal cortical derivation, being restricted to steroidogenic tissues and related tumors. In their study, none of the pheochromocytomas nor the tumors that usually metastasize to the adrenal gland were reactive to SF-1. The diagnostic accuracy of SF-1 was further validated by different groups [50, 51, 52], and this marker represents the best currently available tool in this diagnostic setting.

Variants of adrenal cortical tumors usually have an immunohistochemical profile similar to the conventional type. SF-1, Melan-A, and alpha-inhibin are expressed in oncocytic and myxoid cases in proportions comparable to classic adrenal cortical tumors [17, 51]; neurofilament, either as diffuse cytoplasmic or small paranuclear dots, are more frequently and extensively expressed in the myxoid variant [7]. It is noteworthy that in the sarcomatoid variant of adrenal cortical carcinoma Melan-A, synaptophysin and calretinin are expressed to a lower extent or lost in the sarcomatous areas.

Diagnostic Markers of Malignancy

In the last two decades, several studies have focused on the applicability of immunohistochemical markers as ancillary tools assisting morphology in the diagnosis between benign and malignant lesions. Some of these markers are related to specific genetic alterations occurring more frequently in adrenal cortical carcinomas than in adenomas, whereas others are related to different proliferative profiles within the spectrum of adrenal cortical tumors.

To date, the proliferation marker Ki-67 is the sole immunohistochemical antibody which has steadily been reported useful in the differential diagnosis between adenoma and carcinoma [53–60]. Our group recently proposed phosphohistone H3 immunostaining as an alternative, faster, and reliable method to highlight mitotic figures, being specifically

useful in cases with low mitotic activity [61]. Moreover, in this study, both phospho-histone H3 and Ki-67 immunostainings showed a high interobserver agreement.

Among the variety of other markers reported in the literature, p53 immunoreactivity was found well correlated with the presence of *TP53* gene mutations, which are found in 25–30 % of sporadic adrenal cortical carcinomas, but not in adenomas. For this reason, it is of limited interest as a diagnostic tool because of a good specificity, but a very low sensitivity [60, 62]. IGF-2 protein overexpression has also been observed in a high proportion of adrenal cortical carcinomas and almost no adenomas [60], but it is not usually included in the diagnostic armamentarium because of the difficult interpretation of the labeling.

Prognostic Markers

As described for diagnostic markers, a variety of proteins have been explored as prognostic factors in adrenal cortical carcinoma, either associated with cell proliferation, adrenal cortical differentiation, genetic defects, or specific characteristics of malignant tumor cells. Ki-67 proliferation index has been investigated as a prognostic marker for several years [63, 64]. In a recent study, Ki-67 proved to be superior to mitotic count in terms of prognosis of adrenal cortical carcinoma patients, at least with regard to overall survival, helping to stratify three subgroups of patients based on cutoffs of <20, 20–50, and >50 % [61]. Another promising prognostic marker is SF-1, that, apart from the abovementioned diagnostic role, has also been validated in multiple cohorts as a marker of poor prognosis when expressed at high levels [49, 51]. Possibly providing a molecular explanation for SF-1 protein overexpression in a proportion of adrenal cortical carcinomas, the *NR5A1*, the SF-1 gene, was found amplified in childhood adrenal cortical cancer [47, 48], and chromosomal gains in chromosome 9q (where the SF-1 gene is located) have also been described in adults [65]. Future studies are therefore needed to clarify the prognostic role of *NR5A1* gene amplification in adrenal cortical cancer.

β-Catenin nuclear staining is associated with deregulation of the Wnt/β-catenin signaling pathway and correlates with CTNNB1 (the β-catenin gene) mutations. Its prognostic role has been validated in two independent cohorts of adrenal cortical carcinomas [66]. More recently, Ronchi and coworkers showed that low protein levels of serum glucocorticoid kinase 1, but not of nuclear β-catenin and phosphorylated AKT, were associated with poor overall survival in adrenal cortical carcinoma patients [67].

Several other molecules have also been reported to influence (at the protein expression level) the outcome of adrenal cortical cancer patients, including matrix metalloproteinase type 2 [68] and glucose transporter 1 [69], but have not yet been validated in additional studies.

Genetics of Adrenal Cortical Diseases

Hyperplasia

Some 30 years ago, a peculiar condition was described by Carney at Mayo Clinic, characterized by multiple pigmented adrenal cortical nodules in association with other lesions (myxomas, psammomatous melanotic schwannomas, spotty pigmentation, and blue nevi of the skin or mucosae, together with a variety of endocrine neoplasms) [70]. This seminal description was subsequently confirmed by several additional cases that have led in the past two decades to a better clinical and pathological characterization of this condition as part of the Carney complex. Moreover, different types of mutations of the *PRKAR1A* gene were identified as the genetic causative defect for this syndrome [71].

Adrenal Cortical Tumors in Familial Cancer Susceptibility Syndromes

Even if the majority of adrenal cortical carcinomas arise in a sporadic setting, a minority of cases are associated with familial cancer syndromes, including the autosomal dominant Li–Fraumeni and Beckwith–Wiedemann syndromes and, more rarely, the Gardner syndrome, multiple endocrine neoplasia type 1, neurofibromatosis type 1, and the Carney complex [72] (Table 1). The association of adrenal cortical carcinoma with both the conditions that will subsequently be coded as the Li–Fraumeni and hereditary colon cancer syndromes had already been established in the 1980s [73, 74], but little knowledge on the genetic background was available at that time.

Li–Fraumeni syndrome is a rare autosomal dominant cancer predisposition syndrome associated with germline mutations in the tumor suppressor gene *TP53* and subsequent loss of heterozygosity at the 17p13.1 locus, which confers an increased susceptibility to sarcomas, breast cancer, brain tumors, leukemia, and lymphoma. Adrenal cortical carcinoma, however, develops in only 3–4 % of patients with Li–Fraumeni syndrome, usually in childhood [72]. Common *TP53* mutations include Arg to His substitution at codon 175 (which codes for amino acids of the DNA binding site) and Arg to His at codon 337 (R337H) (coding for the oligomerization domain). The latter is classically observed in children of Southern Brazil [75, 76], where the incidence of adrenal cortical carcinoma is 10 to 15 times higher than in the rest of the world. Screening for germline TP53 mutations in patients with apparently sporadic adrenal cortical carcinoma is recommended, especially in pediatric cases but also in adults, as 4 % of cases were recently reported to bear a germline mutation [77].

In Beckwith–Wiedemann syndrome, various developmental abnormalities, such as macrosomia, exomphalos, macroglossia, abdominal wall defects, ear and renal anomalies, and cleft

Table 1 Summary of most relevant adrenal carcinoma-related inherited susceptibility syndromes

	Li–Fraumeni	Beckwith–Wiedemann	Gardner	MEN1	Carney complex
Tumor	AC	AC	AA (rarely AC)	AA (rarely AC)	AA
Gene/locus	*TP53* (17q13.1)	Alterated imprinting of 11p15.5	*APC* (5q21–22)	*MEN1* (11q13)	*PRKAR1A* (17q23–24)
Protein	p53	IGF-II, p57kip2	APC	Menin	PRKAR1A

AC adrenal carcinoma, *AA* adrenal adenoma

palate, can be associated with pediatric tumors in 5 % of cases, including adrenal cortical carcinoma. Although the majority of cases with Beckwith–Wiedemann syndrome arise de novo, 15 % of them are inherited as the result of a defective genomic imprinting of the 11p15.5 locus. This chromosomal region is usually subjected to a tissue-specific maternal imprinting; therefore, only the paternal allele is expressed [78]. In Beckwith–Wiedemann syndrome, there is a loss of the maternal locus and a gain in the paternal locus. As a consequence, *IGF-2* which is expressed on the paternal allele is overrepresented whereas *p57kip2* and *H19* which are expressed on the maternal allele are defective [79].

Other inherited cancer syndromes associated with adrenal cortical tumors (indeed, more often adenomas than carcinomas and generally restricted to adult patients) are multiple endocrine neoplasia type 1, Gardner syndrome, and neurofibromatosis type 1. In multiple endocrine neoplasia type 1, inactivating mutations in the *MEN1* gene, located in the chromosomal region 11q13, are responsible for the development of pituitary tumors, parathyroid tumors, and other neuroendocrine tumors. In addition, patients are also at risk of developing multiple lipomas, angiomas, and adrenal cortical tumors. The most common adrenal cortical phenotype observed in multiple endocrine neoplasia type 1 is unilateral or bilateral hyperplasia, while adenomas are less common and carcinomas very rare occurrences [80]. Gardner syndrome is an autosomal dominant disorder caused by mutations in the adenomatous polyposis coli (*APC*) gene. Apart from adrenal cortical carcinoma, which is very rare in this syndrome, patients develop gastrointestinal polyps, osteomas, soft tissue tumors, epidermal cysts, desmoid tumors, and periampullary cancer, as well as other endocrine malignancies such as the cribriform variant of papillary thyroid cancer.

Sporadic Adrenal Cortical Tumors

Gene Mutations Inactivating mutations in tumor suppressor genes and activating mutations in oncogenes responsible for familial cancer syndromes have also been found as somatic alterations in sporadic adrenal cortical tumors, with special reference to carcinomas [81]. Losses of the *MEN1* gene locus at 11q13, but very infrequent gene mutations, have been detected in sporadic adrenal tumors [82, 83]. Somatic mutations of the *TP53* gene, as seen in Li–Fraumeni syndrome, as well as p53 protein accumulation can be detected in sporadic tumors and have been considered as a marker of malignancy, being virtually absent in adenomas. Activation of the Wnt/β-catenin pathway as the result of *CTNNB1* mutations has been documented in up to 40 % of carcinomas, but also in a relevant proportion of adenomas [84, 85], especially nonsecreting and/or large-size tumors [86]. Moreover, the presence of activating mutations in the *CTNNB1* gene is associated with a worse outcome in adrenal cortical cancer patients [66]. Finally, somatic inactivating mutations or allelic losses of the *PRKAR1A* locus at 17q22–24, involved in the Carney complex, were also seen in sporadic cases of adrenal cortical adenoma and carcinoma [87].

However, a significant proportion of adrenal cortical tumors lacks known genetic defects, and therefore, several studies have recently been conducted to clarify molecular mechanisms alternative to gene mutations in the pathogenesis of these tumors. Genomic, transcriptomic, and methylomic profiles have been reported in relatively large series of cases and helped to classify adrenal cortical tumor families with even prognostic implications and to distinguish benign from malignant forms. However, an integrative view able to incorporate all such information is still missing, thus making the huge amount of data available still poorly transferable into clinical practice.

Chromosomal Imbalances A number of studies have shown that chromosomal aberrations are more frequent in malignant than in benign and hyperplastic adrenal cortical lesions. Gains, losses, and amplifications can be detected with either comparative genomic hybridization (CGH) or allelotyping techniques. An aneuploid DNA pattern was often associated with such chromosomal imbalances, although the value of DNA ploidy analysis is limited for both diagnostic and prognostic purposes [88]. In particular, gains in chromosomes 6q, 7q, 12q, and 19p, and losses in chromosomes 3, 8, 10p, 16q, 17q, and 19q, have been associated with a significantly worse survival of adrenal cortical cancer patients, independent of tumor size, tumor weight, and functional status of the tumor [89]. A strong relationship between tumor size and number of chromosomal aberrations was reported, with no gains or losses detectable in adenomas smaller than 5 cm; conversely, gains on chromosomes 4 and 5 and losses on 2, 11, and 17

were apparently restricted to carcinomas having a size of 7–20 cm [90]. Overall, extensive genomic imbalances were encountered in carcinomas by means of CGH, indicating that the molecular pathogenesis of sporadic tumors is complex and that multiple genetic changes drive malignant transformation and tumor progression. A recent study on childhood adrenal cortical tumors, using SNP array profiling, identified recurrent alterations in loci comprising well-known oncogenes (*MYC*, *MDM2*, *PDGFRA*, *KIT*, *MCL1*, *BCL2L1*) and tumor suppressors (*TP53*, *RB1*, *RPH3AL*), not yet associated to adrenal cortical carcinoma [91].

Transcriptomic Analysis The last decade has been characterized by the development of high-throughput methods for wide gene expression profiling. Since the first study in 2003 [92], progress has been made in both the understanding of the pathogenesis of adrenal cortical tumors and more recently in the stratification of adrenal cortical carcinomas into prognostic groups.

The early studies aimed at the definition and validation of a malignant signature [93–95]. An initial attempt was made by Slater et al. [95], who classified the tumors into two groups (benign and malignant), according to the Weiss score and identified 74 genes differentially expressed in the two groups. However, by definition, using such an approach, the gene signature of malignancy cannot be a better approach than the Weiss score, which is the reference. To overcome this limitation, other authors used the probability of recurrence as the reference [93]. Among genes of significant impact in this setting, general proliferation markers (cell cycle regulators and cell cycle effectors) common to all cancer types and some adrenal cortical specific markers were identified. Among the latter, the IGF2 gene resulted consistently upregulated in adrenal cortical carcinomas in different transcriptome analyses, thus depicting a specific IGF2 cluster of cases associated also to upregulation of other growth factors and growth factor receptor genes [59, 93, 96–98]. An alternative cluster was characterized by downregulation of steroidogenic enzyme genes, such as *CYP11A*, *CYP11B*, and *HSD3B1* [93], and this cluster was as efficient as pathological evaluation, using a Weiss score cutoff value of 4. Later, deReynies et al. identified two genes, *DGL7* and *PINK1*, as the best molecular predictors of malignancy [97]. All the above observations were then turned into prognostic stratification. Again, by means of unsupervised hierarchical clustering, different authors observed peculiar transcriptome characteristics capable of dividing adrenal cortical carcinomas into cases with bad or good prognosis [96, 97, 99]. In the paper by de Reynies and coworkers [97], the *BUB1B* and *PINK1* genes showed the best prognostic performance. Integrating transcriptome and mutational profiles, three subgroups of adrenal cortical carcinomas with different biological and clinical behavior may be identified: (1) the p53 group, encompassing all tumors with a *TP53* mutation; (2) the β-catenin group, containing all tumors with deregulation of the Wnt/β-catenin pathway (apparently mutually exclusive with p53 group); and (3) the remaining group, with neither p53 nor β-catenin altered pathways but enriched in cell cycle and metabolism genes [100, 101].

MicroRNA Profiling MicroRNAs (miRNAs) are short non-coding RNAs, 18 to 25 nucleotides in length, which influence gene expression either by posttranscriptional regulation of gene expression leading to target mRNA degradation or by the repression of its translation with consequent decrease in the particular protein levels or even by upregulation of their targets [102]. Several studies analyzed the miRNA expression profile in adrenal cortical neoplasms. They mainly aimed at finding out those useful in differentiating adenomas from carcinomas, and to date, a long list of deregulated miRNAs is available [103]. Among them, miR-483 (in both 3p and 5p isoforms) and miR-195 are those more consistently found overexpressed and downregulated, respectively, both at the tissue and serum levels. Data concerning the prognostic role of these two miRNAs are controversial, as they were described as defining a subgroup of carcinomas with a poor prognosis by one group only [104, 105]. miR-210 is another miRNA which was reported upregulated by different groups. It is the miRNA most consistently induced under hypoxia, and high levels were found associated with clinicopathological parameters of aggressiveness (necrosis and high Ki-67 proliferation index) and a poorer survival (Duregon et al., submitted manuscript).

DNA Methylation Profiling The role of DNA hypermethylation in adrenal cortical tumorigenesis has been evaluated in some recent studies. Altered DNA methylation of the H19 promoter had already been shown to be involved in the abnormal expression of both *H19* and *IGF2* genes in adrenal cortical carcinomas [106]. In contrast, the promoter methylation of *TP53* has been demonstrated not to be a significant event in the development of adrenal cortical carcinomas [107]. Recently, a significant DNA hypermethylation of the *RASSF1A* promoter in adrenal cortical carcinoma, but not in adenoma, has been described, suggesting an epigenetic mechanism for *RASSF1A* silencing in malignant adrenal cortical tumors [108]. However, most DNA methylation studies thus far have focused on individual genes. More recently, comprehensive genome-wide analysis of DNA methylation in benign and malignant adrenal cortical tumors has been performed, sometimes obtaining controversial results, possibly related to the different methodological approaches [109–111]. Moreover, Barreau and coworkers distinguished two clusters of adrenal cortical carcinomas based on CpG island methylation status, the CpG island methylator phenotype ("CIMP") and "non-CIMP," the former associated to a poorer prognosis [112].

Adenoma–Carcinoma Sequence

Although there has been much progress in the last 25 years, it is still unclear whether adrenal cortical carcinomas evolve from adenomas following a definite molecular progression pathway. Long-term follow-up of incidentally discovered adrenal cortical neoplasms suggests that adenomas generally maintain a benign phenotype [113]. However, the sequence adenoma–carcinoma has been occasionally postulated in single cases showing the morphological coexistence of an aggressive component embedded within an otherwise adenomatous tissue [114, 115]. In addition, a recent study described a mouse model in which an induced stabilized β-catenin associated with elevated IGF-2 expression resulted in a temporal progression from increasing adrenal cortical hyperplasia to subsequent adenoma, and occasionally carcinoma, formation [116]. Finally, Ronchi et al. recently provided the first genome-wide high-resolution overview of chromosomal changes in large series of adrenal cortical tumors, including adenomas and carcinomas [117]. Among the benign tumors, small isolated copy number gains were the most frequent genetic alterations, and almost all of them were also present in several carcinomas, thus supporting the concept of a common early molecular signature. Moreover, the Wnt/β-catenin and Notch signaling pathways were commonly altered in both adenomas and carcinomas, strengthening their previous hypothesis that these pathways are involved in early tumor pathogenesis.

Future Perspectives: Issues for the Next 25 years

As summarized above, the pathological armamentarium for adrenal cortical disease (and more specifically for tumor characterization) has undergone a wide evolution in the past 25 years. All these achievements in the diagnostic approach and in the phenotypical characterization have allowed a better definition of the disease on the one hand, but have also raised new questions and opportunities for the next 25 years. An arbitrary list of issues to be covered in the near future includes the following:

a. The prospective validation and implementation of the accuracy and reproducibility of the available diagnostic schemes
b. The definition of a grading system for adrenal cortical carcinomas, to further segregate cases associated with "low" and "high" malignant potential
c. The identification and validation of markers predictive of tumor response and progression
d. The identification of novel genetic alterations/pathways involved in adrenal cortical tumorigenesis, with special reference to the subset of cases with no current specific molecular signature
e. The integration of molecular, phenotypic, pathological, and clinical data to design for adrenal cortical tumor patients—as has been done for other human malignancies—individualized clinical management protocols

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References

1. Weiss LM (1984) Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. Am J Surg Pathol 8 (3):163-169
2. Weiss LM, Medeiros LJ, Vickery AL, Jr. (1989) Pathologic features of prognostic significance in adrenocortical carcinoma. Am J Surg Pathol 13 (3):202-206
3. Aubert S, Wacrenier A, Leroy X, Devos P, Carnaille B, Proye C, Wemeau JL, Lecomte-Houcke M, Leteurtre E (2002) Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. Am J Surg Pathol 26 (12):1612-1619
4. Lau SK, Weiss LM (2009) The Weiss system for evaluating adrenocortical neoplasms: 25 years later. Hum Pathol 40 (6):757-768. doi: 10.1016/j.humpath.2009.03.010
5. Blanes A, Diaz-Cano SJ (2007) Histologic criteria for adrenocortical proliferative lesions: value of mitotic figure variability. Am J Clin Pathol 127 (3):398-408. doi: 10.1309/MCGUQ3R4A4WWN3LB
6. Volante M, Bollito E, Sperone P, Tavaglione V, Daffara F, Porpiglia F, Terzolo M, Berruti A, Papotti M (2009) Clinicopathological study of a series of 92 adrenocortical carcinomas: from a proposal of simplified diagnostic algorithm to prognostic stratification. Histopathology 55 (5):535-543. doi: 10.1111/j.1365-2559.2009.03423.x
7. Papotti M, Volante M, Duregon E, Delsedime L, Terzolo M, Berruti A, Rosai J (2010) Adrenocortical tumors with myxoid features: a distinct morphologic and phenotypical variant exhibiting malignant behavior. Am J Surg Pathol 34 (7):973-983. doi:10.1097/PAS.0b013e3181e2b726
8. Duregon E, Volante M, Cappia S, Cuccurullo A, Bisceglia M, Wong DD, Spagnolo DV, Szpak-Ulczok S, Bollito E, Daffara F, Berruti A, Terzolo M, Papotti M (2011) Oncocytic adrenocortical tumors: diagnostic algorithm and mitochondrial DNA profile in 27 cases. Am J Surg Pathol 35 (12):1882-1893. doi:10.1097/PAS.0b013e31822da401
9. Tissier F, Aubert S, Leteurtre E, Al Ghuzlan A, Patey M, Decaussin M, Doucet L, Gobet F, Hoang C, Mazerolles C, Monges G, Renaudin K, Sturm N, Trouette H, Vacher-Lavenu MC, Viallon V, Baudin E, Bertagna X, Coste J, Libe R (2012) Adrenocortical tumors: improving the practice of the Weiss system through virtual microscopy: a National Program of the French Network INCa-COMETE. Am J Surg Pathol 36 (8):1194-1201. doi:10.1097/PAS.0b013e31825a6308
10. Duregon E, Fassina A, Volante M, Nesi G, Santi R, Gatti G, Cappellesso R, Dalino Ciaramella P, Ventura L, Gambacorta M, Dei Tos AP, Loli P, Mannelli M, Mantero F, Berruti A, Terzolo M, Papotti M (2013) The Reticulin Algorithm for Adrenocortical Tumor Diagnosis: A Multicentric Validation Study on 245 Unpublished Cases. Am J Surg Pathol. doi:10.1097/PAS.0b013e31828d387b
11. Kakimoto S, Yushita Y, Sanefuji T, Kondo A, Fujishima N, Kishikawa M, Matsumoto K (1986) Non-hormonal adrenocortical

adenoma with oncocytoma-like appearances. Hinyokika Kiyo 32 (5):757-763
12. Bisceglia M, Ludovico O, Di Mattia A, Ben-Dor D, Sandbank J, Pasquinelli G, Lau SK, Weiss LM (2004) Adrenocortical oncocytic tumors: report of 10 cases and review of the literature. Int J Surg Pathol 12 (3):231-243
13. Wong DD, Spagnolo DV, Bisceglia M, Havlat M, McCallum D, Platten MA (2011) Oncocytic adrenocortical neoplasms—a clinicopathologic study of 13 new cases emphasizing the importance of their recognition. Hum Pathol. doi: 10.1016/j.humpath.2010.08.010
14. Lewis PD, Baxter P, Paul Griffiths A, Parry JM, Skibinski DO (2000) Detection of damage to the mitochondrial genome in the oncocytic cells of Warthin's tumour. J Pathol 191 (3):274-281. doi:10.1002/1096-9896(2000)9999:9999<::AID-PATH634>3.0.CO;2-U
15. Lewis PD, Fradley SR, Griffiths AP, Baxter PW, Parry JM (2002) Mitochondrial DNA mutations in the parotid gland of cigarette smokers and non-smokers. Mutat Res 518 (1):47-54.
16. Tang CK, Harriman BB, Toker C (1979) Myxoid adrenal cortical carcinoma: a light and electron microscopic study. Arch Pathol Lab Med 103 (12):635-638
17. Weissferdt A, Phan A, Suster S, Moran CA (2013) Myxoid adrenocortical carcinoma: a clinicopathologic and immunohistochemical study of 7 cases, including 1 case with lipomatous metaplasia. Am J Clin Pathol 139 (6):780-786. doi:10.1309/AJCPCDZLC13RSXRZ
18. Okazumi S, Asano T, Ryu M, Nagashima T, Odaka M, Isono K, Nishizawa T (1987) [Surgical resection of adrenal carcinoma extending into the vena cava, right atrium and ventricle: case report and review of the literature]. Nippon Geka Gakkai Zasshi 88 (2):231-238
19. Collina G, Maldarizzi F, Betts CM, Eusebi V (1989) Primary sarcomatoid carcinoma of the adrenal gland. First case report. Virchows Arch A Pathol Anat Histopathol 415 (2):161-167
20. Lee MS, Park IA, Chi JG, Ham EK, Lee KC, Lee CW (1997) Adrenal carcinosarcoma—a case report. J Korean Med Sci 12 (4):374-377
21. Sturm N, Moulai N, Laverriere MH, Chabre O, Descotes JL, Brambilla E (2008) Primary adrenocortical sarcomatoid carcinoma: case report and review of literature. Virchows Arch 452 (2):215-219. doi:10.1007/s00428-007-0536-y
22. Coli A, Di Giorgio A, Castri F, Destito C, Marin AW, Bigotti G (2010) Sarcomatoid carcinoma of the adrenal gland: A case report and review of literature. Pathol Res Pract 206 (1):59-65. doi: 10.1016/j.prp.2009.02.012
23. Barksdale SK, Marincola FM, Jaffe G (1993) Carcinosarcoma of the adrenal cortex presenting with mineralocorticoid excess. Am J Surg Pathol 17 (9):941-945
24. Bertolini F, Rossi G, Fiocchi F, Giacometti M, Fontana A, Gibertini MC, Roncucci L, Luppi G, Torricelli P, Rossi A, Conte PF (2011) Primary adrenal gland carcinosarcoma associated with metastatic rectal cancer: a hitherto unreported collision tumor. Tumori 97 (5):27e-30e. doi:10.1700/989.10734
25. Decorato JW, Gruber H, Petti M, Levowitz BS (1990) Adrenal carcinosarcoma. J Surg Oncol 45 (2):134-136
26. Fischler DF, Nunez C, Levin HS, McMahon JT, Sheeler LR, Adelstein DJ (1992) Adrenal carcinosarcoma presenting in a woman with clinical signs of virilization. A case report with immunohistochemical and ultrastructural findings. Am J Surg Pathol 16 (6):626-631
27. Sasaki K, Desimone M, Rao HR, Huang GJ, Seethala RR (2010) Adrenocortical carcinosarcoma: a case report and review of the literature. Diagn Pathol 5:51. doi: 10.1186/1746-1596-5-51
28. Thway K, Olmos D, Shah C, Flora R, Shipley J, Fisher C (2012) Oncocytic adrenal cortical carcinosarcoma with pleomorphic rhabdomyosarcomatous metastases. Am J Surg Pathol 36 (3):470-477. doi:10.1097/PAS.0b013e31824517d9
29. Wieneke JA, Thompson LD, Heffess CS (2003) Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. Am J Surg Pathol 27 (7):867-881
30. Magro G, Esposito G, Cecchetto G, Dall'Igna P, Marcato R, Gambini C, Boldrini R, Collini P, D'Onofrio V, Salfi N, d'Amore E, Ferrari A, Bisogno G, Alaggio R (2012) Pediatric adrenocortical tumors: morphological diagnostic criteria and immunohistochemical expression of matrix metalloproteinase type 2 and human leucocyte-associated antigen (HLA) class II antigens. Results from the Italian Pediatric Rare Tumor (TREP) Study project. Hum Pathol 43 (1):31-39. doi:10.1016/j.humpath.2011.04.016
31. Turk AT, Asad H, Trapasso J, Perilli G, LiVolsi VA (2012) Mixed corticomedullary carcinoma of the adrenal gland: a case report. Endocr Pract 18 (3):e37-42. doi:10.4158/EP11222.CR
32. Lam KY, Lo CY (2002) Metastatic tumours of the adrenal glands: a 30-year experience in a teaching hospital. Clin Endocrinol (Oxf) 56 (1):95-101.
33. Gaffey MJ, Traweek ST, Mills SE, Travis WD, Lack EE, Medeiros LJ, Weiss LM (1992) Cytokeratin expression in adrenocortical neoplasia: an immunohistochemical and biochemical study with implications for the differential diagnosis of adrenocortical, hepatocellular, and renal cell carcinoma. Hum Pathol 23 (2):144-153
34. Jorda M, De MB, Nadji M (2002) Calretinin and inhibin are useful in separating adrenocortical neoplasms from pheochromocytomas. Appl Immunohistochem Mol Morphol 10 (1):67-70
35. Browning L, Bailey D, Parker A (2008) D2-40 is a sensitive and specific marker in differentiating primary adrenal cortical tumours from both metastatic clear cell renal cell carcinoma and phaeochromocytoma. J Clin Pathol 61 (3):293-296. doi: 10.1136/jcp.2007.049544
36. Schroder S, Niendorf A, Achilles E, Dietel M, Padberg BC, Beisiegel U, Dralle H, Bressel M, Kloppel G (1990) Immunocytochemical differential diagnosis of adrenocortical neoplasms using the monoclonal antibody D11. Virchows Arch A Pathol Anat Histopathol 417 (2):89-96
37. Schroder S, Padberg BC, Achilles E, Holl K, Dralle H, Kloppel G (1992) Immunocytochemistry in adrenocortical tumours: a clinicomorphological study of 72 neoplasms. Virchows Arch A Pathol Anat Histopathol 420 (1):65-70
38. Tartour E, Caillou B, Tenenbaum F, Schroder S, Luciani S, Talbot M, Schlumberger M (1993) Immunohistochemical study of adrenocortical carcinoma. Predictive value of the D11 monoclonal antibody. Cancer 72 (11):3296-3303
39. Komminoth P, Roth J, Schroder S, Saremaslani P, Heitz PU (1995) Overlapping expression of immunohistochemical markers and synaptophysin mRNA in pheochromocytomas and adrenocortical carcinomas. Implications for the differential diagnosis of adrenal gland tumors. Lab Invest 72 (4):424-431
40. Busam KJ, Iversen K, Coplan KA, Old LJ, Stockert E, Chen YT, McGregor D, Jungbluth A (1998) Immunoreactivity for A103, an antibody to melan-A (Mart-1), in adrenocortical and other steroid tumors. Am J Surg Pathol 22 (1):57-63
41. Ghorab Z, Jorda M, Ganjei P, Nadji M (2003) Melan A (A103) is expressed in adrenocortical neoplasms but not in renal cell and hepatocellular carcinomas. Appl Immunohistochem Mol Morphol 11 (4):330-333
42. Arola J, Liu J, Heikkila P, Voutilainen R, Kahri A (1998) Expression of inhibin alpha in the human adrenal gland and adrenocortical tumors. Endocr Res 24 (3-4):865-867
43. McCluggage WG, Burton J, Maxwell P, Sloan JM (1998) Immunohistochemical staining of normal, hyperplastic, and neoplastic adrenal cortex with a monoclonal antibody against alpha inhibin. J Clin Pathol 51 (2):114-116
44. Munro LM, Kennedy A, McNicol AM (1999) The expression of inhibin/activin subunits in the human adrenal cortex and its tumours. J Endocrinol 161 (2):341-347.

45. Arola J, Liu J, Heikkila P, Ilvesmaki V, Salmenkivi K, Voutilainen R, Kahri AI (2000) Expression of inhibin alpha in adrenocortical tumours reflects the hormonal status of the neoplasm. J Endocrinol 165 (2):223-229.
46. Sasano H, Shizawa S, Suzuki T, Takayama K, Fukaya T, Morohashi K, Nagura H (1995) Transcription factor adrenal 4 binding protein as a marker of adrenocortical malignancy. Hum Pathol 26 (10): 1154-1156
47. Figueiredo BC, Cavalli LR, Pianovski MA, Lalli E, Sandrini R, Ribeiro RC, Zambetti G, DeLacerda L, Rodrigues GA, Haddad BR (2005) Amplification of the steroidogenic factor 1 gene in childhood adrenocortical tumors. J Clin Endocrinol Metab 90 (2):615-619. doi: 10.1210/jc.2004-0942
48. Pianovski MA, Cavalli LR, Figueiredo BC, Santos SC, Doghman M, Ribeiro RC, Oliveira AG, Michalkiewicz E, Rodrigues GA, Zambetti G, Haddad BR, Lalli E (2006) SF-1 overexpression in childhood adrenocortical tumours. Eur J Cancer 42 (8):1040-1043. doi: 10.1016/j.ejca.2006.01.022
49. Sbiera S, Schmull S, Assie G, Voelker HU, Kraus L, Beyer M, Ragazzon B, Beuschlein F, Willenberg HS, Hahner S, Saeger W, Bertherat J, Allolio B, Fassnacht M (2010) High diagnostic and prognostic value of steroidogenic factor-1 expression in adrenal tumors. J Clin Endocrinol Metab 95 (10):E161-171. doi: 10.1210/jc.2010-0653
50. Sangoi AR, Fujiwara M, West RB, Montgomery KD, Bonventre JV, Higgins JP, Rouse RV, Gokden N, McKenney JK (2011) Immunohistochemical distinction of primary adrenal cortical lesions from metastatic clear cell renal cell carcinoma: a study of 248 cases. Am J Surg Pathol 35 (5):678-686. doi:10.1097/PAS.0b013e3182152629
51. Duregon E, Volante M, Giorcelli J, Terzolo M, Lalli E, Papotti M (2013) Diagnostic and prognostic role of steroidogenic factor 1 in adrenocortical carcinoma: a validation study focusing on clinical and pathologic correlates. Hum Pathol 44 (5):822-828. doi:10.1016/j.humpath.2012.07.025
52. Weissferdt A, Phan A, Suster S, Moran CA (2013) Adrenocortical Carcinoma: A Comprehensive Immunohistochemical Study of 40 Cases. Appl Immunohistochem Mol Morphol. doi:10.1097/PAI.0b013e31828a96cf
53. McNicol AM, Struthers AL, Nolan CE, Hermans J, Haak HR (1997) Proliferation in Adrenocortical Tumors: Correlation with Clinical Outcome and p53 Status. Endocr Pathol 8 (1):29-36.
54. Iino K, Sasano H, Yabuki N, Oki Y, Kikuchi A, Yoshimi T, Nagura H (1997) DNA topoisomerase II alpha and Ki-67 in human adrenocortical neoplasms: a possible marker of differentiation between adenomas and carcinomas. Mod Pathol 10 (9): 901-907
55. Nakazumi H, Sasano H, Iino K, Ohashi Y, Orikasa S (1998) Expression of cell cycle inhibitor p27 and Ki-67 in human adrenocortical neoplasms. Mod Pathol 11 (12):1165-1170
56. Arola J, Salmenkivi K, Liu J, Kahri AI, Heikkila P (2000) p53 and Ki67 in adrenocortical tumors. Endocr Res 26 (4):861-865
57. Terzolo M, Boccuzzi A, Bovio S, Cappia S, De Giuli P, Ali A, Paccotti P, Porpiglia F, Fontana D, Angeli A (2001) Immunohistochemical assessment of Ki-67 in the differential diagnosis of adrenocortical tumors. Urology 57 (1):176-182.
58. Stojadinovic A, Brennan MF, Hoos A, Omeroglu A, Leung DH, Dudas ME, Nissan A, Cordon-Cardo C, Ghossein RA (2003) Adrenocortical adenoma and carcinoma: histopathological and molecular comparative analysis. Mod Pathol 16 (8):742-751. doi:10.1097/01.MP.0000081730.72305.81
59. Soon PS, Gill AJ, Benn DE, Clarkson A, Robinson BG, McDonald KL, Sidhu SB (2009) Microarray gene expression and immunohistochemistry analyses of adrenocortical tumors identify IGF2 and Ki-67 as useful in differentiating carcinomas from adenomas. Endocr Relat Cancer 16 (2):573-583. doi:10.1677/ERC-08-0237
60. Schmitt A, Saremaslani P, Schmid S, Rousson V, Montani M, Schmid DM, Heitz PU, Komminoth P, Perren A (2006) IGFII and MIB1 immunohistochemistry is helpful for the differentiation of benign from malignant adrenocortical tumours. Histopathology 49 (3):298-307. doi: 10.1111/j.1365-2559.2006.02505.x
61. Duregon E, Molinaro L, Volante M, Ventura L, Righi L, Bolla S, Terzolo M, Sapino A, Papotti M (2013) Comparative diagnostic and prognostic performances of morphological and phospho-histone H3-based mitotic count and Ki-67 proliferation index in adrenocortical carcinoma. Mod Pathol. (in press)
62. Libe R, Groussin L, Tissier F, Elie C, Rene-Corail F, Fratticci A, Jullian E, Beck-Peccoz P, Bertagna X, Gicquel C, Bertherat J (2007) Somatic TP53 mutations are relatively rare among adrenocortical cancers with the frequent 17p13 loss of heterozygosity. Clin Cancer Res 13 (3):844-850. doi: 10.1158/1078-0432.CCR-06-2085
63. Vargas MP, Vargas HI, Kleiner DE, Merino MJ (1997) Adrenocortical neoplasms: role of prognostic markers MIB-1, P53, and RB. Am J Surg Pathol 21 (5):556-562
64. Morimoto R, Satoh F, Murakami O, Suzuki T, Abe T, Tanemoto M, Abe M, Uruno A, Ishidoya S, Arai Y, Takahashi K, Sasano H, Ito S (2008) Immunohistochemistry of a proliferation marker Ki67/MIB1 in adrenocortical carcinomas: Ki67/MIB1 labeling index is a predictor for recurrence of adrenocortical carcinomas. Endocr J 55 (1):49-55.
65. Dohna M, Reincke M, Mincheva A, Allolio B, Solinas-Toldo S, Lichter P (2000) Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. Genes Chromosomes Cancer 28 (2):145-152. doi:10.1002/(SICI)1098-2264(200006)28:2<145::AID-GCC3>3.0.CO;2-7
66. Gaujoux S, Grabar S, Fassnacht M, Ragazzon B, Launay P, Libe R, Chokri I, Audebourg A, Royer B, Sbiera S, Vacher-Lavenu MC, Dousset B, Bertagna X, Allolio B, Bertherat J, Tissier F (2011) Beta-catenin activation is associated with specific clinical and pathologic characteristics and a poor outcome in adrenocortical carcinoma. Clin Cancer Res 17 (2):328-336. doi: 10.1158/1078-0432.CCR-10-2006
67. Ronchi CL, Sbiera S, Leich E, Tissier F, Steinhauer S, Deutschbein T, Fassnacht M, Allolio B (2012) Low SGK1 Expression in Human Adrenocortical Tumors Is Associated with ACTH-Independent Glucocorticoid Secretion and Poor Prognosis. J Clin Endocrinol Metab. doi: 10.1210/jc.2012-2669
68. Volante M, Sperone P, Bollito E, Frangipane E, Rosas R, Daffara F, Terzolo M, Berruti A, Papotti M (2006) Matrix metalloproteinase type 2 expression in malignant adrenocortical tumors: Diagnostic and prognostic significance in a series of 50 adrenocortical carcinomas. Mod Pathol 19 (12):1563-1569. doi: 10.1038/modpathol.3800683
69. Fenske W, Volker HU, Adam P, Hahner S, Johanssen S, Wortmann S, Schmidt M, Morcos M, Muller-Hermelink HK, Allolio B, Fassnacht M (2009) Glucose transporter GLUT1 expression is an stage-independent predictor of clinical outcome in adrenocortical carcinoma. Endocr Relat Cancer 16 (3):919-928. doi: 10.1677/ERC-08-0211
70. Carney JA, Hruska LS, Beauchamp GD, Gordon H (1986) Dominant inheritance of the complex of myxomas, spotty pigmentation, and endocrine overactivity. Mayo Clin Proc 61 (3):165-172
71. Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA (2000) Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. Nat Genet 26 (1):89-92. doi:10.1038/79238
72. Else T (2012) Association of adrenocortical carcinoma with familial cancer susceptibility syndromes. Mol Cell Endocrinol 351 (1):66-70. doi:10.1016/j.mce.2011.12.008
73. Painter TA, Jagelman DG (1985) Adrenal adenomas and adrenal carcinomas in association with hereditary adenomatosis of the colon and rectum. Cancer 55 (9):2001-2004

74. Lynch HT, Kimberling W, Albano WA, Lynch JF, Biscone K, Schuelke GS, Sandberg AA, Lipkin M, Deschner EE, Mikol YB, et al. (1985) Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). I. Clinical description of resource. Cancer 56 (4):934-938
75. Achatz MI, Hainaut P, Ashton-Prolla P (2009) Highly prevalent TP53 mutation predisposing to many cancers in the Brazilian population: a case for newborn screening? Lancet Oncol 10 (9):920-925. doi:10.1016/S1470-2045(09)70089-0
76. Custodio G, Parise GA, Kiesel Filho N, Komechen H, Sabbaga CC, Rosati R, Grisa L, Parise IZ, Pianovski MA, Fiori CM, Ledesma JA, Barbosa JR, Figueiredo FR, Sade ER, Ibanez H, Arram SB, Stinghen ST, Mengarelli LR, Figueiredo MM, Carvalho DC, Avilla SG, Woiski TD, Poncio LC, Lima GF, Pontarolo R, Lalli E, Zhou Y, Zambetti GP, Ribeiro RC, Figueiredo BC (2013) Impact of neonatal screening and surveillance for the TP53 R337H mutation on early detection of childhood adrenocortical tumors. J Clin Oncol 31 (20):2619-2626. doi:10.1200/JCO.2012.46.3711
77. Herrmann LJ, Heinze B, Fassnacht M, Willenberg HS, Quinkler M, Reisch N, Zink M, Allolio B, Hahner S (2012) TP53 germline mutations in adult patients with adrenocortical carcinoma. J Clin Endocrinol Metab 97 (3):E476-485. doi:10.1210/jc.2011-1982
78. DeChiara TM, Robertson EJ, Efstratiadis A (1991) Parental imprinting of the mouse insulin-like growth factor II gene. Cell 64 (4):849-859.
79. Weksberg R, Shuman C, Smith AC (2005) Beckwith-Wiedemann syndrome. Am J Med Genet C Semin Med Genet 137C (1):12-23. doi:10.1002/ajmg.c.30058
80. Langer P, Cupisti K, Bartsch DK, Nies C, Goretzki PE, Rothmund M, Roher HD (2002) Adrenal involvement in multiple endocrine neoplasia type 1. World J Surg 26 (8):891-896. doi:10.1007/s00268-002-6492-4
81. Bertherat J, Bertagna X (2009) Pathogenesis of adrenocortical cancer. Best Pract Res Clin Endocrinol Metab 23 (2):261-271. doi:10.1016/j.beem.2008.10.006
82. Heppner C, Reincke M, Agarwal SK, Mora P, Allolio B, Burns AL, Spiegel AM, Marx SJ (1999) MEN1 gene analysis in sporadic adrenocortical neoplasms. J Clin Endocrinol Metab 84 (1):216-219
83. Schulte KM, Mengel M, Heinze M, Simon D, Scheuring S, Kohrer K, Roher HD (2000) Complete sequencing and messenger ribonucleic acid expression analysis of the MEN I gene in adrenal cancer. J Clin Endocrinol Metab 85 (1):441-448
84. Tissier F, Cavard C, Groussin L, Perlemoine K, Fumey G, Hagnere AM, Rene-Corail F, Jullian E, Gicquel C, Bertagna X, Vacher-Lavenu MC, Perret C, Bertherat J (2005) Mutations of beta-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors. Cancer Res 65 (17):7622-7627. doi: 10.1158/0008-5472.CAN-05-0593
85. Berthon A, Martinez A, Bertherat J, Val P (2012) Wnt/beta-catenin signalling in adrenal physiology and tumour development. Mol Cell Endocrinol 351 (1):87-95. doi:10.1016/j.mce.2011.09.009
86. Bonnet S, Gaujoux S, Launay P, Baudry C, Chokri I, Ragazzon B, Libe R, Rene-Corail F, Audebourg A, Vacher-Lavenu MC, Groussin L, Bertagna X, Dousset B, Bertherat J, Tissier F (2011) Wnt/beta-catenin pathway activation in adrenocortical adenomas is frequently due to somatic CTNNB1-activating mutations, which are associated with larger and nonsecreting tumors: a study in cortisol-secreting and -nonsecreting tumors. J Clin Endocrinol Metab 96 (2): E419-426. doi: 10.1210/jc.2010-1885
87. Yu B, Ragazzon B, Rizk-Rabin M, Bertherat J (2012) Protein kinase A alterations in endocrine tumors. Horm Metab Res 44 (10):741-748. doi:10.1055/s-0032-1316292
88. Cibas ES, Medeiros LJ, Weinberg DS, Gelb AB, Weiss LM (1990) Cellular DNA profiles of benign and malignant adrenocortical tumors. Am J Surg Pathol 14 (10):948-955
89. Stephan EA, Chung TH, Grant CS, Kim S, Von Hoff DD, Trent JM, Demeure MJ (2008) Adrenocortical carcinoma survival rates correlated to genomic copy number variants. Mol Cancer Ther 7 (2):425-431. doi:10.1158/1535-7163.MCT-07-0267
90. Kjellman M, Kallioniemi OP, Karhu R, Hoog A, Farnebo LO, Auer G, Larsson C, Backdahl M (1996) Genetic aberrations in adrenocortical tumors detected using comparative genomic hybridization correlate with tumor size and malignancy. Cancer Res 56 (18):4219-4223
91. Letouze E, Rosati R, Komechen H, Doghman M, Marisa L, Fluck C, de Krijger RR, van Noesel MM, Mas JC, Pianovski MA, Zambetti GP, Figueiredo BC, Lalli E (2012) SNP array profiling of childhood adrenocortical tumors reveals distinct pathways of tumorigenesis and highlights candidate driver genes. J Clin Endocrinol Metab 97 (7):E1284-E1293. doi:10.1210/jc.2012-1184
92. Giordano TJ, Thomas DG, Kuick R, Lizyness M, Misek DE, Smith AL, Sanders D, Aljundi RT, Gauger PG, Thompson NW, Taylor JM, Hanash SM (2003) Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. Am J Pathol 162 (2):521-531. doi: 10.1016/S0002-9440(10)63846-1
93. de Fraipont F, El Atifi M, Cherradi N, Le Moigne G, Defaye G, Houlgatte R, Bertherat J, Bertagna X, Plouin PF, Baudin E, Berger F, Gicquel C, Chabre O, Feige JJ (2005) Gene expression profiling of human adrenocortical tumors using complementary deoxyribonucleic acid microarrays identifies several candidate genes as markers of malignancy. J Clin Endocrinol Metab 90 (3):1819-1829. doi: 10.1210/jc.2004-1075
94. Velazquez-Fernandez D, Laurell C, Geli J, Hoog A, Odeberg J, Kjellman M, Lundeberg J, Hamberger B, Nilsson P, Backdahl M (2005) Expression profiling of adrenocortical neoplasms suggests a molecular signature of malignancy. Surgery 138 (6):1087-1094. doi: 10.1016/j.surg.2005.09.031
95. Slater EP, Diehl SM, Langer P, Samans B, Ramaswamy A, Zielke A, Bartsch DK (2006) Analysis by cDNA microarrays of gene expression patterns of human adrenocortical tumors. Eur J Endocrinol 154 (4):587-598. doi: 10.1530/eje.1.02116
96. Giordano TJ, Kuick R, Else T, Gauger PG, Vinco M, Bauersfeld J, Sanders D, Thomas DG, Doherty G, Hammer G (2009) Molecular classification and prognostication of adrenocortical tumors by transcriptome profiling. Clin Cancer Res 15 (2):668-676. doi: 10.1158/1078-0432.CCR-08-1067
97. de Reynies A, Assie G, Rickman DS, Tissier F, Groussin L, Rene-Corail F, Dousset B, Bertagna X, Clauser E, Bertherat J (2009) Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. J Clin Oncol 27 (7):1108-1115. doi: 10.1200/JCO.2008.18.5678
98. Tombol Z, Szabo PM, Molnar V, Wiener Z, Tolgyesi G, Horanyi J, Riesz P, Reismann P, Patocs A, Liko I, Gaillard RC, Falus A, Racz K, Igaz P (2009) Integrative molecular bioinformatics study of human adrenocortical tumors: microRNA, tissue-specific target prediction, and pathway analysis. Endocr Relat Cancer 16 (3):895-906. doi:10.1677/ERC-09-0096
99. Laurell C, Velazquez-Fernandez D, Lindsten K, Juhlin C, Enberg U, Geli J, Hoog A, Kjellman M, Lundeberg J, Hamberger B, Larsson C, Nilsson P, Backdahl M (2009) Transcriptional profiling enables molecular classification of adrenocortical tumours. Eur J Endocrinol 161 (1):141-152. doi:10.1530/EJE-09-0068
100. Assie G, Giordano TJ, Bertherat J (2012) Gene expression profiling in adrenocortical neoplasia. Mol Cell Endocrinol 351 (1):111-117. doi:10.1016/j.mce.2011.09.044
101. de Krijger RR, Papathomas TG (2012) Adrenocortical neoplasia: evolving concepts in tumorigenesis with an emphasis on adrenal cortical carcinoma variants. Virchows Arch 460 (1):9-18. doi:10.1007/s00428-011-1166-y

102. Rana TM (2007) Illuminating the silence: understanding the structure and function of small RNAs. Nat Rev Mol Cell Biol 8 (1):23-36. doi: 10.1038/nrm2085
103. Singh P, Soon PS, Feige JJ, Chabre O, Zhao JT, Cherradi N, Lalli E, Sidhu SB (2012) Dysregulation of microRNAs in adrenocortical tumors. Mol Cell Endocrinol 351 (1):118-128. doi:10.1016/j.mce.2011.09.041
104. Soon PS, Tacon LJ, Gill AJ, Bambach CP, Sywak MS, Campbell PR, Yeh MW, Wong SG, Clifton-Bligh RJ, Robinson BG, Sidhu SB (2009) miR-195 and miR-483-5p Identified as Predictors of Poor Prognosis in Adrenocortical Cancer. Clin Cancer Res 15 (24):7684-7692. doi: 10.1158/1078-0432.CCR-09-1587
105. Chabre O, Libe R, Assie G, Barreau O, Bertherat J, Bertagna X, Feige JJ, Cherradi N (2013) Serum miR-483-5p and miR-195 are predictive of recurrence risk in adrenocortical cancer patients. Endocr Relat Cancer 20 (4):579-594. doi:10.1530/ERC-13-0051
106. Gao ZH, Suppola S, Liu J, Heikkila P, Janne J, Voutilainen R (2002) Association of H19 promoter methylation with the expression of H19 and IGF-II genes in adrenocortical tumors. J Clin Endocrinol Metab 87 (3):1170-1176
107. Sidhu S, Martin E, Gicquel C, Melki J, Clark SJ, Campbell P, Magarey CJ, Schulte KM, Roher HD, Delbridge L, Robinson BG (2005) Mutation and methylation analysis of TP53 in adrenal carcinogenesis. Eur J Surg Oncol 31 (5):549-554. doi: 10.1016/j.ejso.2005.01.013
108. Korah R, Healy JM, Kunstman JW, Fonseca AL, Ameri AH, Prasad ML, Carling T (2013) Epigenetic silencing of RASSF1A deregulates cytoskeleton and promotes malignant behavior of adrenocortical carcinoma. Mol Cancer 12:87. doi:10.1186/1476-4598-12-87
109. Fonseca AL, Kugelberg J, Starker LF, Scholl U, Choi M, Hellman P, Akerstrom G, Westin G, Lifton RP, Bjorklund P, Carling T (2012) Comprehensive DNA methylation analysis of benign and malignant adrenocortical tumors. Genes Chromosomes Cancer 51 (10):949-960. doi:10.1002/gcc.21978
110. Rechache NS, Wang Y, Stevenson HS, Killian JK, Edelman DC, Merino M, Zhang L, Nilubol N, Stratakis CA, Meltzer PS, Kebebew E (2012) DNA methylation profiling identifies global methylation differences and markers of adrenocortical tumors. J Clin Endocrinol Metab 97 (6):E1004-E1013. doi:10.1210/jc.2011-3298
111. Liu-Chittenden Y, Kebebew E (2013) CpG island methylator phenotype in adrenocortical carcinoma: fact or fiction? J Clin Endocrinol Metab 98 (1):48-50. doi:10.1210/jc.2012-4063
112. Barreau O, Assie G, Wilmot-Roussel H, Ragazzon B, Baudry C, Perlemoine K, Rene-Corail F, Bertagna X, Dousset B, Hamzaoui N, Tissier F, de Reynies A, Bertherat J (2013) Identification of a CpG island methylator phenotype in adrenocortical carcinomas. J Clin Endocrinol Metab 98 (1):E174-184. doi:10.1210/jc.2012-2993
113. Barzon L, Sonino N, Fallo F, Palu G, Boscaro M (2003) Prevalence and natural history of adrenal incidentalomas. Eur J Endocrinol 149 (4):273-285
114. Bernard MH, Sidhu S, Berger N, Peix JL, Marsh DJ, Robinson BG, Gaston V, Le Bouc Y, Gicquel C (2003) A case report in favor of a multistep adrenocortical tumorigenesis. J Clin Endocrinol Metab 88 (3):998-1001
115. Trezzi R, Poli F, Fellegara G (2009) "Dedifferentiated" adrenal cortical neoplasm. Int J Surg Pathol 17 (4):343-344. doi:10.1177/1066896909335155
116. Heaton JH, Wood MA, Kim AC, Lima LO, Barlaskar FM, Almeida MQ, Fragoso MC, Kuick R, Lerario AM, Simon DP, Soares IC, Starnes E, Thomas DG, Latronico AC, Giordano TJ, Hammer GD (2012) Progression to adrenocortical tumorigenesis in mice and humans through insulin-like growth factor 2 and beta-catenin. Am J Pathol 181 (3):1017-1033. doi:10.1016/j.ajpath.2012.05.026
117. Ronchi CL, Sbiera S, Leich E, Henzel K, Rosenwald A, Allolio B, Fassnacht M (2013) Single nucleotide polymorphism array profiling of adrenocortical tumors—evidence for an adenoma carcinoma sequence? PLoS One 8 (9):e73959. doi:10.1371/journal.pone.0073959