Adrenocortical cancer: pathophysiology and clinical management

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Abstract

Adrenocortical cancer (ACC) is a rare tumor with a poor prognosis. By contrast, benign adrenocortical tumors are frequent, underlying the importance of a correct diagnosis of malignancy of such tumors. ACC can be diagnosed by the investigation of endocrine signs of steroid excess, symptoms due to tumor growth or an adrenal incidentaloma. Hormonal investigations demonstrate in most ACC steroid oversecretion, the dominant characteristics being a co-secretion of cortisol and androgens. Imaging by CT-scan or MRI shows a large heterogeneous tumor with a low fat content. Careful pathological investigation with the assessment of the Weiss score is important for the diagnosis of malignancy. Molecular markers can also be helpful and in the future might be important for prognosis. Tumors localized to the adrenal gland (McFarlane stages 1 and 2) have a better outcome than invasive and metastatic tumors (stages 3 and 4). Tumor removal by a specialized team is crucial for treatment and should always aim at complete removal. In patients with metastatic or progressive disease, medical treatment is started with mitotane that requires a close monitoring of its blood level. Surgery is indicated when possible for local recurrence but also in some cases of metastasis. Local treatment (radiofrequency, chemoembolization, and radiation therapy) can have some indications for metastatic disease. In patients with disease progression cytotoxic chemotherapy can be used. Despite the best care, the overall prognosis of ACC is poor with a 5-year survival rate below 30% in most series. Therefore, progress in the understanding of the pathophysiology of ACC is important. Despite the rarity of ACC, significant advances have been made in the understanding of its pathogenesis the last decade. These progresses came mainly from the study of the genetics of ACC, both at the germline level in rare familial diseases, and at the somatic level by the study of molecular alterations in sporadic tumors. These advances underline the importance of genetic alterations in ACC development and point-out to various chromosomal regions (2, 11p15, 11q, 17p13) and genes (IGF-II, p53, β-catenin, ACTH receptor). This review will summarize these advances as well as the current clinical management of ACC.

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Introduction

Adrenocortical tumors, mostly benign adenomas, are frequent in the general population and nowadays most often found incidentally (Grumbach et al. 2003). By contrast, adrenocortical cancer (ACC) is rare, with an estimated prevalence between 4 and 12 per million in adults (Grumbach et al. 2003). Despite this rarity, malignancy is always feared because of its poor prognosis when investigating an adrenocortical mass (Luton et al. 2000). The diagnosis of malignancy of adrenocortical tumors relies on careful investigations of clinical, biological and imaging features before surgery and pathological examination after tumor removal.
Progress in the understanding of the pathophysiology of ACC is important to improve diagnosis, prognosis evaluation, and treatment. This review will summarize the advances in the molecular mechanisms of adrenocortical tumors development that have recently been made and the current clinical management of ACC.

Pathophysiology of adrenocortical cancer

The analysis of tumor clonality is an important step to establish the cellular origin of neoplasms and to identify the mechanisms underlying tumor progression. Monoclonality indicates that tumor progression is the end result of an intrinsic genetic mutation, whereas polyclonality suggests that tumor cells are affected by local or systemic stimuli. Analysis of the pattern of X-chromosome inactivation in heterozygous female tissue has shown that ACC consists of monoclonal populations of cells, whereas benign tumors might be monoclonal as well as polyclonal (Beuschlein et al. 1994, Gicquel et al. 1994).

Monoclonal tumors result from genetic alterations conferring a growth advantage to the cell initially affected. These genetic events can be studied at the scale of the whole genome, as losses or gains of part or all of a chromosome. A large number of molecular techniques, such as comparative genomic hybridization (CGH) and microsatellite analysis, can be used in genome-wide screen for such chromosomal alterations. These approaches have identified alterations affecting various chromosomes and loci. Interestingly, a positive correlation has been observed between tumor size and the number of CGH changes in adrenocortical tumors, suggesting that chromosomal alterations accumulate during tumor progression (Sidhu et al. 2002). It was demonstrated by CGH that chromosomal alterations are observed in 28% of benign adrenocortical tumors (Kjellman et al. 1996). Most of the changes observed concern losses on chromosomes 2, 11q and 17p and gains on chromosomes 4 and 5 (Kjellman et al. 1999, Zhao et al. 1999, Dohna et al. 2000, Sidhu et al. 2002).

In more recent studies, CGH identified changes in 61% of benign tumors and the most common gains observed were on chromosomes 5, 12, 19, and 4 (Sidhu et al. 2002). Losses were observed at 1p, 17p, 22p, 22q, 2q, and 11q in up to 62% of cases of ACC. Studies using microsatellite markers have demonstrated a high percentage of loss of heterozygosity (LOH) or allelic imbalance at 11q13 (≥90%), 17p13 (≥85%), and 2p16 (92%) in ACC (Kjellman et al. 1999, Gicquel et al. 2001).

The genes involved in these molecular alterations could be classified as tumor suppressor genes on one hand, and oncogenes on the other hand. Molecular alterations would lead to inactivation of the tumor suppressor genes and activation of the oncogenes. This simple way to classify the various alterations involved in oncogenesis will be used in this paragraph to review the pathophysiology of ACC.

Oncogenes

IGF-II (insulin-like growth factor II)

The IGF-II gene located at 11p15 encodes an important fetal growth factor, is maternally imprinted and is therefore expressed only from the paternal allele (DeChiara et al. 1991; Fig. 1). The 11p15 region is

![Figure 1](https://www.endocrinology-journals.org/)

**Figure 1** Alterations of 11p15 locus and IGF-II overexpression in ACC. The imprinted 11p15 locus contains the CDKN1C (p57kip2), IGF-II, and H19 genes. In normal differentiated tissue (on the left), only the paternal allele of the IGF-II gene is expressed, whereas only the maternal alleles of CDKN1C and H19 are expressed. Paternal isodisomy is usually observed in adrenal cancers (on the right) with loss of the maternal allele at 11p15. This leads to the overexpression of IGF-II and decreased expression of CDKN1C and H19. According to the studies performed in cells lines, IGF-II could act as an autocrine growth factor through binding to the IGF-I receptor on adrenocortical cancer cells.
organized into two different clusters: a telomeric domain including the IGF-II gene (DeChiara et al. 1991), H19 (Hao et al. 1993) and a centromeric domain including CDKNIC (p57kip2; Lee et al. 1995, Matsuoka et al. 1995). The H19 mRNA is not translated and this gene may modulate IGF-II expression. The p57kip2 gene encodes a cyclin-dependent kinase inhibitor involved in the G1/S phase of the cell cycle. The H19 and p57kip2 genes are paternally imprinted and are therefore expressed from the maternal allele only. Genetic or epigenetic changes in the imprinted 11p15 region, resulting in increases in IGF-II expression, and mutations of the p57kip2 gene have been implicated in Beckwith–Wiedemann syndrome (Lam et al. 1999). This overgrowth disorder is characterized by macrosomia, macroglossia, organomegaly, and developmental abnormalities (in particular, abdominal wall defects with exomphalos), embryonal tumors, such as Wilms’ tumor – and ACC (Wiedmann 1983, Hertel et al. 2003), neuroblastoma, and hepatoblastoma.

IGF-II mRNA is efficiently translated and malignant tumors contain large amounts of IGF-II protein, some of which is in the prohormone form. The insulin-like growth factors system is involved in the development of the adrenal cortex and its role has been largely documented in adrenocortical tumors (Mesiano et al. 1993, Gicquel et al. 1995, 2001). Many studies have demonstrated that IGF-II is strongly overexpressed in malignant adrenocortical tumors, with such overexpression observed in approximately 90% of ACC (Ilvesmaki et al. 1993a, Gicquel et al. 1997, Boulle et al. 1998; Fig. 2). Transcriptome analysis of adrenocortical tumors has demonstrated that IGF-II is the gene most overexpressed in ACC by comparison with benign adrenocortical adenomas or normal adrenal glands (Giordano et al. 2003, Bourdeau et al. 2004, de Fraipont et al. 2005) The mechanisms underlying IGF-II overexpression are paternal isodisomy (loss of the maternal allele and duplication of the paternal allele) or, less frequently, loss of imprinting (Ogawa et al. 1993, Rainier et al. 1993); with maintenance of both parental alleles but a paternal-like IGF-II gene expression pattern (Gicquel et al. 1997).

Receptors for IGF-I and IGF-II are present in adrenal tissues and strong overexpression of intact IGF-I receptors has been shown in ACC (Weber et al. 1997a). The mitogenic effect of IGF-II is dependent on the IGF-I receptor, as reported by Logie et al. (1999), who demonstrated that IGF-II is involved in the H295R cell line (derived from an ACC) proliferation and acts through the IGF-I receptor. IGF-II effects are restricted to tumors and plasma IGF-II concentrations are usually in the normal range. The biological effects of IGFs are modulated in vivo by six IGF-binding proteins (IGFBPs), which positively or negatively regulate the effects of IGFs, depending on their abundance and affinity for growth factors. H295R cells and adrenocortical tumors with IGF-II overproduction have been shown to contain large amounts of IGFBP-2 (Boulle et al. 1998), suggesting that IGFBP-2 may regulate IGF-II effects in ACC. Furthermore, IGFBP-2 levels have been shown to correlate with tumor stage in ACC.

![Figure 2](image-url)
(Bouillé et al. 2000), In ACC, only the maternal H19 allele is expressed, so expression of this gene is abolished in most ACC displaying paternal isodisomy (Gicquel et al. 1997). Methylation of the H19 promoter has been shown to be involved in the abnormal expression of both H19 and IGF-II in human ACC (Gao et al. 2002). Expression of p57kip2 is also abolished in ACC (Liu et al. 1997), but the precise role of the product encoded by this gene in the cell cycle machinery and tumorigenesis requires confirmation. Chromosome region 11p15 LOH is associated with a higher risk of tumor recurrence, is more frequent in ACC than in adrenal adenomas (78.5 vs 9.5%) and correlates with Weiss score (a score of cytopathological alterations used for the diagnosis of malignancy, see below; Gicquel et al. 2001). Thus, 11p15 alterations could be used as a biological marker for predicting ACC malignancy after surgical removal of the tumor (Gicquel et al. 2001). However, 11p15 LOH seems to have a lower predictive value than 17p13 LOH.

\( \textcolor{red}{\text{β-Catenin activation in ACC}} \)

Genetic alterations of the Wnt signaling pathway were initially identified in familial adenomatous polyposis coli and have been extended to a variety of cancers (Kikuchi 2003). Adrenocortical tumors have been observed in some case reports of patients with familial adenomatous polyposis coli (Naylor & Gardner 1981). Furthermore, familial adenomatous polyposis coli patients with germline mutations of the APC (Adenomatous Polyposis Coli) gene that lead to an activation of the Wnt signaling pathway, may develop ACTs (Blaker et al. 2004). Molecular studies have suggested that somatic mutations of APC could occur in these tumors in patients already having a germline defect.

The Wnt signaling pathway is normally activated during embryonic development. β-Catenin is a key component of this signaling pathway. It has a structural role in cell–cell adhesion, and is a transcription cofactor with T-cell factor/lymphoid enhancer factor (TCF/LEF) mediating transcriptional activation of target genes of the Wnt signaling pathway (Fig. 3).

Interestingly, gene profiling studies in various types of adrenocortical tumors have shown the frequent activation of Wnt signaling target genes: in ACC, a microarray approach has shown that ectodermal-neural cortex-1 (ECN-1) was up-regulated (Giordano et al. 2003). In both benign and malignant ACT, β-catenin accumulation can be observed. These alterations seem very frequent in ACC, consistent with an abnormal activation of the Wnt-signaling pathway. This is explained in a subset of adrenocortical tumors by genetic alterations of the Wnt signaling pathway, may develop ACTs (Blaker et al. 2004). Molecular studies have suggested that somatic mutations of APC could occur in these tumors in patients already having a germline defect.

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\( \textcolor{red}{\text{RAS oncogene}} \)

Ras proteins are membrane associated proteins involved in downstream signaling, once ligand stimulation of growth factor receptor occurs. The three ras proteins (H, N, and K) are one of the most commonly mutated oncogenes in human cancers (Shields et al. 2000). Controversial data are present in the literature: Lin et al. (1998) found K-ras mutations in about 50% of tumor tissues of Conn’s adenomas and no mutations are observed in H-ras, while Moul et al. (1993) and Ocker et al. (2000) did not identify Ras mutations.
Growth factors

Various growth factors and cytokines other than IGFs have been shown to regulate adrenal growth and function in normal adult and fetal adrenals. These include basic fibroblast growth factor (FGF-2), transforming growth factor-α (TGF-α) and transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), and interleukins (Hotta & Baird 1986, Feige et al. 1991, 1998, Ilvesmaki et al. 1993b, Weber et al. 1997a,b, de Fraipont et al. 2000, Turner et al. 2003). Among these factors, FGF-2 may be a prime candidate to evaluate in adrenocortical tumors. This growth factor is highly expressed in adrenal tissues and is one of the most potent mitogens in cell culture of adult and fetal adrenal (Mesiano et al. 1991, Feige et al. 1998). In human fetal adrenal glands, Mesiano et al. (1993) showed a cooperative mitogenic effect of IGF-II and FGF-2. Boulle et al. (2000) demonstrated that FGF-2 is mitogenic for the H295R cells, regulates the expression of both IGF-II and IGFBP-2, and modulates the processing of pro-IGF-II.

Angiogenesis plays a major role in cancer growth and metastasis. The angiogenic status of a tumor can be assessed by the study of VEGF expression. In ACC, an overexpression of VEGF by comparison with adrenal adenomas has been observed (de Fraipont et al. 2000, Bernini et al. 2002). However, a dissociation between a high expression of VEGF and a low vascularization was observed in ACC, suggesting a dissociation between the angiogenic status and the neoangiogenic capabilities of these tumors (Bernini et al. 2002). Very interestingly, serum VEGF levels were significantly higher in patients with ACC than in patients with adrenal adenomas and normal subjects (Kolomecki et al. 2001). Although a significant reduction of serum VEGF levels 1 month after surgery of ACC has been reported (Kolomecki et al. 2000), its use as a tumor marker remains to be investigated.

Transforming growth factor-β1 (TGF-β1), another multifunctional growth modulator, inhibits the proliferation of epithelial cells and regulates adult and fetal adrenal growth and functions. Two different studies demonstrated a reduced TGF-β1 mRNA expression in ACC, while no difference in the expression (nor mutation) of TGF-β1 receptor were observed in ACC (Boccuzzi et al. 1999, Arnaldi et al. 2000).

Tumor suppressor genes

TP53

The tumor suppressor gene TP53 is located at 17p13 and involved in the control of cell proliferation. Acquired mutations of the TP53 gene are common tumor-specific alterations in humans, and have been identified in most of the major types of cancer (Caron de Fromentel & Soussi 1992). Germline mutations in TP53 are identified in 70% of families with Li-Fraumeni syndrome (LFS). This syndrome displays dominant inheritance and confers susceptibility to breast carcinoma, soft tissue sarcoma, brain tumors, osteosarcoma, leukemia, and ACC (Hisada et al. 1998). Other possible component tumors include melanoma, gonadal germ cells tumors, and carcinoma of the lung, pancreas, and prostate. These tumors have an early onset, affecting mostly children and young adults. Mutations in checkpoint kinase 2 gene (hCHK2), encoding a kinase that can directly phosphorylate TP53, have been reported in LFS patients (Bell et al. 1999). However, in these few kindreds there is no report of ACC (Bell et al. 1999). Germline mutations in TP53 have been observed in 50–80% of children with apparently sporadic ACC in North America and Europe (Wagner et al. 1994, Varley et al. 1999). The incidence of pediatric ACC is about 10 times higher in Southern Brazil than in the rest of the world, and a specific germline mutation has been identified in exon 10 of the TP53 gene (R337H) in almost all cases (Latronico et al. 2001, Ribeiro et al. 2001). Molecular studies about this mutation have shown that its tissue-specific effects may be pH-dependent, due to the replacement of an arginine by a histidine in the tetramerization domain of TP53 (DiGiammarino et al. 2002).

In sporadic ACC in adults, somatic mutations of TP53 are found in only 25% of cases and are located in four ‘hot spot regions’ within exons 5 and 8, as first demonstrated by Ohgaki et al. (1993) and Reincke et al. (1994) in a small series. An Italian group recently reported a TP53 mutation rate of 70% in 10 ACC (Barzon et al. 2001). Lin et al. (1994) reported TP53 mutations in 73% of adrenocortical adenomas from Taiwanese patients, with 82% of these mutations located in exon 4. Reincke et al. (1996) sequenced exon 4 of TP53 in 19 adrenocortical adenomas from Caucasian patients but found no mutation; they suggested that environmental factors might account for this discrepancy.

LOH at 17p13 has been consistently demonstrated in ACC but not in adrenocortical adenomas (Yano et al. 1989, Gicquel et al. 2001; Fig. 2). LOH at 17p13 was recently reported to occur in 85% of malignant tumors and less than 30% of benign adenomas. LOH at 17p13 is correlated with the Weiss score. It has therefore been suggested that 17p13 LOH could be used as a molecular marker of malignancy in adrenocortical tumors.
In a large prospective study of patients with ACT, 17p13 LOH was demonstrated to be an independent variable predictive of recurrence after complete surgical removal of localized adrenocortical tumors (Gicquel et al. 2001).

The discrepancy between the frequencies of TP53 mutation and 17p13 LOH may be accounted for by the existence of another tumor suppressor gene in this region. The HIC-1 gene (hypermethylated in cancer) is such a candidate. It encodes a transcription factor triggered by TP53 and inactivated by hypermethylation or allelic losses in various cancers (Wales et al. 1995).

**MEN 1 gene**

The MEN 1 gene, located at the 11q13 locus, is thought to be a tumor suppressor gene. A heterozygous inactivating germline mutation of MEN 1 is found in about 90% of families affected by multiple endocrine neoplasia type 1 (MEN 1). The principal clinical features of this autosomal dominant syndrome include parathyroid (95%), endocrine pancreas (45%) and pituitary (45%) tumors, and thymic carcinoids (Thakker 1998). Adrenocortical tumors and/or hyperplasia are observed in 25–40% of MEN 1 patients (Kjellman et al. 1999, Schulte et al. 2000). In most cases, they are non-functional adrenocortical adenomas. Hyperplasia is typically found in MEN 1 patients presenting ACTH hypersecretion (Cushing’s disease), whereas ACC has rarely been reported in MEN 1 patients. Somatic mutation of the MEN 1 gene is very rare in adrenocortical tumors (Heppner et al. 1999, Schulte et al. 2000). By contrast, LOH at 11q13 are observed in more than 90% of informative ACC and only 20% of adrenocortical adenomas (Heppner et al. 1999, Kjellman et al. 1999, Schulte et al. 2000). However, LOH in ACC involves almost all the 11q domain, suggesting that an as yet unidentified tumor suppressor gene located on the long arm of the chromosome is involved in ACC formation.

**PRKAR1A**

The regulatory R1A subunit of protein kinase A (PRKAR1A) is a key component of the cAMP signaling pathway that has been implicated in endocrine tumorigenesis (Bertherat 2001, Bossis & Stratakis 2004). This gene, that maps at the 17q22-24 locus, is implicated in a dominantly multiple neoplasia inherited syndrome, the Carney complex (CNC; Kirschner et al. 2000a,b), characterized by spotty skin pigmentation (lentiginosis), endocrine overactivity with primary pigmented nodular adrenocortical disease (PPNAD) and cardiac myxomas (Carney et al. 1985, Groussin et al. 2002a,b, 2006). Heterozygous inactivating germline mutations of PRKAR1A have been demonstrated in about 45 to 65% of CNC families (Kirschner et al. 2000b, Veugelers et al. 2004). Somatic PRKAR1A mutations have been demonstrated in sporadic secreting adrenocortical adenomas, with clinical, hormonal, and pathological characteristics similar to those of PPNAD (Bertherat et al. 2003).

LOH at 17q22-24 has been also observed in sporadic adrenocortical adenomas and seems to be restricted to the PRKAR1A locus, suggesting the possible involvement of this tumor suppressor gene. By contrast, LOH seems to affect a large part of 17q in ACC, suggesting that PRKAR1A alteration may play only a minor role in malignant adrenocortical tumor growth.

**ACTH receptor (ACTH-R)**

ACTH-R belongs to a subgroup of five receptors of the G-protein-coupled receptors superfamily. It is encoded by an intron-less gene on chromosome 18p11.2. ACTH-R LOH has been observed in two of four informative ACC, but not in 15 hypersecreting adrenocortical adenomas, suggesting a role for ACTH-R in cellular differentiation (Reincke et al. 1997). ACTH-R expression studied by Northern blot or in situ hybridization seems up-regulated in functional adrenocortical adenomas. By contrast, a low ACTH-R mRNA level, suggesting down-regulation of the receptor, is observed in non-functional adrenocortical adenomas and ACC (Reincke et al. 1997, 1998). Moreover, Fassnacht et al. (1998) demonstrated that aminoglutethimide, an inhibitor of glucocorticoids synthesis, induces profound ACTH-R down-regulation in the human H295 adrenocortical carcinoma cell line, either by altering the gene expression or by decreasing transcript accumulation through an effect on RNA stability.

**Clinical management of adrenocortical cancer**

**Epidemiology**

ACC is a rare disease with an estimated incidence between 1 and 2 per million and per year in adults in North America and Europe (Soreide et al. 1992, Lindholm et al. 2001). In children, the incidence is considered as ten times lower except in South Brazil where there is a higher incidence of pediatric ACC, recently explained by a specific germline p53 mutation as discussed above (Ribeiro et al. 2001). There is in most series an increased female to male ratio (Hutter & Kayhoe 1966, Luton et al. 2000, Icard et al. 2001),
although not always reported (Venkatesh et al. 1989). The prevalence of ACC in female patients with Cushing syndrome diagnosed during pregnancy is higher than in non-pregnant patients (Guilhaume et al. 1992).

**Diagnosis of adrenocortical cancer**

*Clinical and hormonal investigations*

Symptoms leading to the diagnosis of adrenalcortical cancer (ACC) can be due to hormone hypersecretion and/or tumor mass and metastasis (Luton et al. 1990, Abiven et al. 2006). Although ACC are rare among incidentalomas, the diagnosis of ACC is nowadays more often made during the diagnostic work-up of an adrenal incidentaloma (Luton et al. 2000). This circumstance is important since it might be a way to diagnose an ACC at an earlier stage and to improve the prognosis (Abiven et al. 2006). Other specific feature that may be associated with rare genetic diseases, such as the Li-Fraumeni and Wiedemann–Beckwith syndromes, where ACC is part of a more complex syndrome as discussed above.

The proportion of secreting tumors among ACC varies in the literature from one-quarter to three-quarters (Didolkar et al. 1981, Abiven et al. 2006). This could be due to the differences in hormonal investigations and/or recruitment bias. It seems that the majority of ACC are usually secreting tumors when careful hormonal investigations are performed (MacFarlane 1958, Kasperlik-Zaluska et al. 1995, Favia et al. 2001, Abiven et al. 2006). By contrast with benign adrenocortical tumors (that usually secrete a single class of steroid), ACC can secrete various types of steroids (see Table 1). Co-secretion of androgens and cortisol is the most frequent and highly suggestive of a malignant adrenocortical tumor (Luton et al. 1990, Wajchenberg et al. 2000, Allolio et al. 2004, Abiven et al. 2006). Cortisol oversecretion will induce centripetal obesity, protein wasting with skin thinning and striae, muscle atrophy (myopathy), and osteoporosis. Cortisol excess can also cause impaired defense against infection, diabetes, hypertension, psychiatric disturbances, and gonadal dysfunction in men and women. Androgen oversecretion may induce various manifestations in women: hirsutism, menstrual abnormalities, infertility, and eventually frank virilisation (alopecia, deepening of the voice, clitoris hypertrophy). ACC can also secrete mineralocorticoids and steroids precursors. Oversecretion of estrogens can be observed in rare cases. Estrogen excess is responsible for gynecomastia in males. Routine hormonal investigations therefore aim at the characterization of the steroid secretory profile of ACC. Steroid excess diagnosis is useful to establish the adrenocortical origin of the tumor and can be used for follow-up. ACTH-independent cortisol oversecretion is easily demonstrable (Newell-Price et al. 2006); increased urinary cortisol excretion that is not suppressible with high doses of dexamethasone, and undetectable ACTH plasma levels. Plasma 17-OH progesterone is often elevated (baseline and/or after ACTH stimulation), as well as the specific adrenal androgen dehydroepiandrosterone (DHEA)-S which leads to increased plasma testosterone in females. Other steroids as compound S, DOC, Delta 4 androstenedione, and estradiol can be overproduced by the tumor. Secretion of aldosterone by ACC is not frequent and can be detected by plasma aldosterone and renin assays. Probably less than a third of ACCs are ‘non-hypersecretory’ after careful hormonal investigations. In these cases, one should be cautious not to overdiagnose a tumor of the adrenal area as an ACC. These non-hypersecretory ACCs can be diagnosed after investigation of adrenal incidentalomas or discovered by the manifestations of the tumor growth or extension: local symptoms (pain, palpation of a tumor, venous thrombosis, etc.), or distant metastases (liver, lung, and bones). Fever may occur, concomitant to tumor necrosis. However, the general condition of the patient is most often preserved except at a very late stage when the tumor is non-secreting. It explains that non-hypersecretory ACCs may be diagnosed at a late stage.

**Table 1 Hormonal investigations in patients with ACC**

<table>
<thead>
<tr>
<th>Glucocorticoid secretion (a minimum of three tests)</th>
<th>24 h urinary free cortisol and urinary creatinine</th>
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<tbody>
<tr>
<td>Dexamethasone suppression test (1 mg)</td>
<td>Basal ACTH</td>
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<tr>
<td>Basal cortisol</td>
<td>Sex steroids</td>
</tr>
<tr>
<td>Testosterone (in female)</td>
<td>Estradiol (in male and post-menopausal women)</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>17-OH-progesterone</td>
</tr>
<tr>
<td>DHEA-S (or DHEA)</td>
<td>S-compound</td>
</tr>
<tr>
<td>Precursors</td>
<td>Desoxycorticosterone</td>
</tr>
<tr>
<td>17-OH-progesterone</td>
<td>Mineralocorticoids</td>
</tr>
<tr>
<td>S-compound</td>
<td>Aldosterone/renin ratio (patients with hypertension or hypokalemia)</td>
</tr>
</tbody>
</table>

These assays are adapted from the recommendation of the ACC working group of the European Network for the Study of Adrenal Tumors (ENSAT), the steroids in italics are not part of the minimal ENSAT work-up. This imply prior exclusion of a pheochromocytoma by the assay of urinary or plasma meta- and normetanephrine.
Imaging investigations
Imaging is an essential diagnostic step for ACC, especially in cases of incidentaloma. It is important for both the diagnosis of malignancy of an adrenal mass and the extension work-up. Adrenal computed tomography scan (CT-scan) is a very informative imaging procedure for adrenocortical tumors (Boland et al. 1998, Hamrahian et al. 2005). In ACC, it shows a unilateral mass, that is most often large (above 5–6 cm, typically 10 cm and above), lowering the kidney. A part from the size of the tumor, the features suggestive of malignancy are the lack of homogeneity with foci of necrosis and irregular margins; a high spontaneous density observed before contrast media injection during CT-scan (above 10 HU), indicating a low fat density observed before contrast media injection (above 10 HU), indicating a low fat content by opposition to what is often found in adenomas. When spontaneous density is above 10 HU, dynamic measurement of contrast-enhanced adenomas. When spontaneous density is above 10 HU, dynamic measurement of contrast-enhanced densities may give additional information to distinguish between benign and malignant lesions. The wash-out after contrast media injection during CT-scan is typically below 50% in ACC (Pena et al. 2000). CT-scan also participates in the detection of local invasion and distant metastases (liver, lung). Loco-regional vessel invasion through the renal veins and the inferior vena cava can proceed up to the right atrium and result in metastatic lung embolism (Icard et al. 2001). Adrenal scintigraphy (Gross et al. 1994) with iodocholesterol is not routinely needed. It might help in some situations since adrenocortical adenomas usually give a positive scintigraphy. By contrast in ACC, especially non-secreting ones, a negative scintigraphic result can be observed (Gross et al. 1994). MRI and/or ultra sound also participate in the diagnosis of liver nodules, and venous invasions. Bone scintigraphy may help to evaluate bone metastases. However, in patients with Cushing’s syndrome, bone remodeling and/or fracture can induce false positive results of bone scintigraphy.

More recently studies have demonstrated that ACCs almost invariably have a high up take of 18-fluorodesoxyglucose ((18)-FDG). Thus, (18)-FDG PET scan appears to distinguish between benign and malignant adrenal tumors (Becherer et al. 2001, Maurea et al. 2001, Tenenbaum et al. 2004). This simple, non-traumatic imaging procedure also participates in the extension work-up (Lebouleux et al. 2006). PET using (11)C-labeled metomidate is a tissue-specific imaging procedure that has been demonstrated to detect both adrenal adenomas and ACC (Hennings et al. 2006). Its use in the extension work-up of ACC needs to be investigated.

Pathology and molecular analysis
As often with endocrine tumors, the diagnosis of malignancy of adrenocortical lesions is not always easy for the pathologist. There is not a single pathological feature which will lead to the diagnosis of a malignant adrenal cortical tumor. Combinations of various histological parameters allowing the calculation of a ‘score’ for a given tumor have been developed. The most widely used is the Weiss score, which is made of nine different items. Each item is given a value of one, when it is present, and zero when it is absent. The score is obtained by summing the values of each individual item. Since the initial paper of Weiss (1984), it is assumed that a score above 3 is most likely to be associated with a malignant tumor. However, there is often a strong doubt for malignancy for scores at 3 and even in some rare cases (Pohlink et al. 2004). Since the Weiss score has limitations and it is dependant on the experience of the pathologist, there is an effort to develop molecular markers of malignancy. As described previously, IGF-II overexpression and allelic losses at 17p13 have been suggested as useful markers (Gicquel et al. 2000, Libé & Bertherat 2005). Immunohistochemistry of Cyclin E or Ki-67 that are higher in malignant adrenocortical tumors has also been suggested in the literature as potential useful tools (Terzolo et al. 2001, Tissier et al. 2004).

Prognosis of adrenocortical cancer
Among the various clinical parameters that have been shown to impact on ACC prognosis, tumor staging has been demonstrated as one of the most important. The MacFarlane staging (MacFarlane 1958), modified by Sullivan et al. (1978) is the most commonly used and relies on surgical finding and extension work-up. Four different stages are differentiated with this score. Stages 1 and 2 tumors are localized to the adrenal cortex and present a maximum diameter below or above 5 cm respectively. Locally invasive tumors or tumors with regional lymph node metastases are classified as stage 3, whereas stage 4 consists of tumors invading adjacent organs or presenting with distant metastases. The prognosis of stages 1 and 2 tumors is better than that of stages 3 or 4 tumors (Icard et al. 2001, Abiven et al. 2006; Fig. 4). A better survival is usually reported in younger patients (Luton et al. 1990, Abiven et al. 2006). Cortisol secreting tumor is associated with a worse prognosis (Berruti et al. 2005, Abiven et al. 2006). This could be due to the morbidity associated with Cushing’s syndrome and/or to a different tumor progression. Some pathological features, such as a high mitotic rate or
atypic mitotic figures have been shown to be associated with a poor prognosis (Stojadinovic et al. 2002).

In the future, it is expected that molecular tools will give a better prediction of the prognosis of ACC. Gene profiling approach can already differentiate malignant from benign tumors (Giordano et al. 2003, de Fraipont et al. 2005). Preliminary results suggest that it might also be of value in determining the prognosis of malignant tumors (de Fraipont et al. 2005).

Treatment of adrenocortical cancer

Surgery and local therapy

Surgery of the adrenal tumor is the major treatment of stage 1–3 ACC (Fig. 5). It can also be discussed in stage 4 patients. Only complete tumor removal can lead to long-term remission (Icard et al. 2001, Schteingart et al. 2005, Abiven et al. 2006). Open adrenalectomy is currently recommended as laparoscopic removal of malignant adrenocortical tumors could be associated with a high risk of peritoneal dissemination (Cobb et al. 2005). Substitutive glucocorticoid therapy should be started after surgery of cortisol-secreting tumors to avoid adrenal deficiency. In stage 4 patients, with distant metastases, tumor, debulking with removal of the primary adrenal tumor can be discussed in order to improve both prognosis and reduce steroid excess. One should note that if reduction of steroid excess by partial tumor removal is obvious, no prospective trial investigating the effect on survival has been reported. However, tumor debulking might also help to improve the results of other therapeutic options. When the number of metastases is limited their surgical removal can also be discussed. Radiofrequency thermal ablation of liver and lung metastasis below 4–5 cm of maximal diameter can be an alternative to surgical removal (Wood et al. 2003). Chemoembolization has also been used for liver metastasis (de Baere 2006). Surgery of bone metastasis can be indicated to reduce fracture risk, or, in case of spinal localization, neurological symptoms.

Radiation therapy

Radiation therapy is usually considered as not very effective to control tumor growth. However, it has been recently suggested that tumor bed radiation therapy could help to prevent local recurrence after surgical removal (Allolio & Fassnacht in press). For bone metastasis, radiotherapy can be used as a palliative treatment to reduce pain and limit the risk of development of local complications (neurological symptoms and/or fracture).

Medical therapy with mitotane

When complete tumor removal is not possible, or in case of recurrence, medical treatment with O,p’DDD (ortho, para’, dichloro-, diphenyl-, dichloro ethane, or mitotane) is recommended (Luton et al. 1990, Wooten and King, 1993). It has both an anticortisolic action and inhibits steroid synthesis by an action on steroidogenic enzymes, as 11β-hydroxylase and cholesterol side chain cleavage. It is quite specific of the adrenal cortex. Interestingly, O,p’DDD has also a cytotoxic effect on the adrenocortical cells that is important for its use in ACC. It is usually effective to control steroid excess in patients with secreting ACC. Most series reported in the literature on the efficacy of O,p’DDD in ACC are retrospective analysis with variable results on tumor progression. A recent review suggests that an objective tumor regression could be observed in 25% of the cases (Allolio & Fassnacht 2006). We have recently shown in a retrospective study, that patients with cortisol secreting ACC have a better survival rate when starting treatment with mitotane (O,p’DDD) 3 months following the surgery of the adrenal tumor (Abiven et al. 2006). However, the effect of mitotane after complete removal of MacFarlane stage 1 or 2 tumors has never been studied in prospective trials. Considering the very poor prognosis of ACC it might be discussed in patients with bad prognostic factors as an adjuvant treatment after complete tumor removal. This issue is important and randomized trials are needed for its clarification.

A mitotane blood level of at least 14 mg/l seems to improve the tumor response rate (van Slooten et al. 1984, Baudin et al. 2001). However, the side effects of mitotane (mainly digestive and neurological) often
limit the ability to reach this suggested optimal level. The daily mitotane dose required to achieve this 14 mg/l level varies from patients to patients. Therefore, close monitoring of mitotane blood level is very helpful to remain in the narrow range between 14 and 20 mg/l, considered by most authors as the therapeutic range of mitotane in ACC. Since O,p’DDD can induce adrenal insufficiency, substitutive glucocorticoid and mineralocorticoid therapy should be associated.

Medical treatment with cytotoxic chemotherapy
Several cytotoxic chemotherapy regimens have been used in ACC. They are usually considered in patients with tumor progression under mitotane therapy reaching the plasma blood level of 14 mg/l or presenting severe side effects limiting its use. Various drugs have been used and the experience is still limited. It is currently accepted since the Ann Arbor international conference on ACC (Schteingart et al. 2005), that the
combined treatment with cis-platine, etoposide, doxorubicin (EDP regimen) associated with O,p’DDD (Berruti et al. 2005) and streptozotocin also given with O,p’DDD (Khan et al. 2000) are the better regimens. The EDP regimen consists of 4 days of treatment repeated every 28 days and for each cycle the following dose of each drug is given: doxorubicine: 40 mg/m², etposide: 300 mg/m², and cisplatine 80 mg/m² (Berruti et al. 2005). An international trial first inter national randomized trial in locally advanced and metastatic adrenocortical carcinoma treatment (FIRM- ACT) is currently done to investigate the results of these two treatments (Allolio & Fassnacht 2006).

Conclusion

Considering the rarity of ACC, significant advances reviewed herein have been made this last decade to understand its pathophysiology. These advances have been also important for a better diagnosis of these tumors and might ultimately lead to a better prediction of prognosis. However, there is need for much more progress, especially in improving therapeutic efficiency (Kirschner 2006). Due to the rarity of ACC, collaborative work performed in national and international networks dedicated to adrenocortical tumors will be important. In Europe, this will be the goal of the European Network for the Study of Adrenal tumors (ENSAT) which has been recently developed on the background of several national networks already working successfully in this field.

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