Clinical Cancer Research

β-Catenin Activation Is Associated with Specific Clinical and Pathologic Characteristics and a Poor Outcome in Adrenocortical Carcinoma

Sébastien Gaujoux[1–3], Sophie Grabar[4,5], Martin Fassnacht[6], Bruno Ragazzon[1,2], Pierre Launay[1,2,9], Rossella Libé[1,2,7,8], Ilham Chokri[9], Anne Audebourg[9], Benedict Royer[9], Silviu Sbiera[6], Marie-Cécile Vacher-Lavenu[5,9], Bertrand Dousset[1–3,5], Xavier Bertagna[1,2,5,7,8], Bruno Allolio[6], Jérôme Bertherat[1,2,5,7,8], and Frédérique Tissier[1,2,5,7,9]

Abstract

Purpose: Activation of the Wnt/β-catenin signaling pathway is frequent in adrenocortical carcinoma (ACC) and might be associated with a more aggressive phenotype. The objective of this study was to assess the prognostic value of β-catenin immunohistochemistry and *CTNNB1* (β-catenin gene)/*APC* (adenomatous polyposis coli gene) mutations in patients with resected primary ACC.

Experimental design: In 79 patients with resected primary ACC from a French cohort (Cochin-COMETE), β-catenin expression was assessed on tumor specimens by immunohistochemistry. For patients with available DNA ($n = 49$), *CTNNB1*, and *APC hotspot* (mutation cluster region), were sequenced. Association between these results and the clinicopathologic characteristics of the ACC and overall and disease-free survival were studied. Results were confirmed on a tissue microarray from an independent multicentric cohort of 92 ACC from Germany (German-ENSAT cohort).

Results: In the Cochin-COMETE cohort, the presence of a β-catenin nuclear staining was significantly associated with a higher ENSAT tumor stage (i.e., stages III and IV), higher Weiss score, more frequent necrosis, mitoses, and *CTNNB1*/*APC* mutations. β-Catenin nuclear staining and the presence of *CTNNB1*/*APC* mutations were both associated with decreased overall and disease-free survival, and were independent predictive factors of survival in multivariate analysis. The same results were observed in the German-ENSAT cohort.

Conclusions: Wnt/β-catenin activation, confirmed by the presence of β-catenin nuclear staining, is an independent prognostic factor of overall and disease-free survival in patients with resected primary ACC.
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Introduction

Adrenocortical carcinoma (ACC) is a rare (1, 2) and highly aggressive endocrine neoplasm with a 5-year overall survival around 40% (3, 4). Complete initial surgery remains the only curative option for these patients, and adjuvant treatment is of limited effectiveness. To date, the only widely accepted and clinically used prognostic factor is the Macfarlane–Sullivan classification (5, 6), with its recent proposed modification by the ENSAT (European Network for the Study of Adrenal Tumors) group (3). Nevertheless, the outcome of ACC patients with resected primary tumors seem to be heterogeneous within this classification (7, 8), and this variability is likely to reflect different biological behavior and tumorigenesis patterns, as previously shown by gene expression profiling analysis (9, 10). Therefore, tools based on tumor biology would be expected to be reliable and relevant prognostic factors, in addition to pathologic examination and tumor staging (9, 10).

Several observations support the importance of the activation of the Wnt/β-catenin signaling pathway in adrenal cortex tumorigenesis (11–13), as previously shown in a variety of human cancers (14–17). In animal models, a constitutive activation of the Wnt/β-catenin pathway in the adrenal cortex of transgenic mice leads to the development of adrenocortical tumors with malignant characteristics (18). In humans, gene profiling studies have reported overexpression of target genes of the Wnt/β-catenin signaling pathway in ACC (19), and it appears that this pathway might play an important role through β-catenin gene

Authors' Affiliations: [1]Cochin Institute, Paris Descartes University, CNRS (UMR 8104), [2]Inserm, U1016, [3]AP-HP, Cochin Hospital, Department of Digestive and Endocrine Surgery, [4]AP-HP, Cochin Hospital, Unit of Biostatistic and Epidemiology, [5]Paris Descartes University, Paris, France; [6]University of Wuerzburg, University Hospital, Department of Internal Medicine I, Endocrine and Diabetes Unit, 97080 Wuerzburg, Germany; [7]Rare Adrenal Cancer Network-Corticomédullosurrénale Tumeur Endocrine, Institut National du Cancer, [8]AP-HP, Cochin Hospital, Department of Endocrinology, Center for Rare Adrenal Diseases, and [9]AP-HP, Cochin Hospital, Department of Pathology, Paris, France

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Corresponding Author: Frédérique Tissier, Hôpital Cochin—Service d'anatomie et cytologie pathologiques, 27, rue du Faubourg Saint-Jacques, 75679 Paris cedex 14; France. Phone: 33 1 58 41 15 93; Fax: 33 1 58 41 14 80; E-mail: frederique.tissier@cch.aphp.fr.

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Translational Relevance

Adrenocortical carcinoma (ACC) is a rare and highly aggressive endocrine neoplasm, with an overall poor prognosis. Activation of the Wnt/β-catenin pathway is frequent in ACC, and we herein showed with 2 independent cohorts that activation of this pathway is associated with a specific clinical and pathologic pattern and a decreased overall and disease-free survival. Those results highlight the importance of this pathway for a better understanding of ACC pathogenesis, and its potential implication in the management of ACC, as a prognostic factor and/or as a future target for therapy.

(*CTNNB1*) mutations (11, 13). This is emphasized by a frequent cytoplasmic and/or nuclear β-catenin accumulation shown by immunohistochemistry, revealing activation of the Wnt/β-catenin signaling pathway (11). It has been hypothesized that activation of this pathway could drive a tumor toward a particular phenotype. Indeed, activation of the Wnt/β-catenin pathway and the presence of a *CTNNB1* mutation have recently been associated in primary pigmented nodular adrenocortical disease (PPNAD) and ACC with a more aggressive tumorigenesis (12). All these findings suggest that the activation of the Wnt/β-catenin signaling pathway might have a specific impact on ACC biology and therefore tumor outcome.

We studied 2 large independent cohorts of patients with resected primary ACC (the Cochin-COMETE cohort and the German-ENSAT cohort) to assess the influence of the activation of the Wnt/β-catenin signaling pathway, indicated by nuclear β-catenin accumulation and/or the presence of a *CTNNB1/APC* (adenomatous polyposis coli) mutation, on clinical and pathologic characteristics and outcome of ACC.

Patients, Materials, and Methods

Patients and tissue collection

Cochin-COMETE cohort. From January 1988 to December 2007, 79 patients with resected primary ACC who followed up at the Cochin Hospital were enrolled. For each patient, the diagnosis was confirmed using adrenal tumor tissue removed during surgery by an experienced pathologist (FT), and only tumors with a Weiss score more than or equal to 3 were included (20, 21). The clinical, hormonal, and radiological investigations were performed as previously reported (22–24). Surgery was indicated when R0 resection was supposed possible on preoperative work-up, whatever the tumor stage. Staging was performed using ENSAT's criteria (3). After surgery, patients were examined twice a year for 2 years and annually thereafter, until their date of death or loss to follow-up, as previously reported (24). For 49 patients, fragments of tumors obtained during surgery were immediately dissected, frozen, and stored in liquid nitrogen until use, and were available for nucleic acid extraction and mutation analysis. Informed signed consent for genetic diagnosis, tumor analysis, and for access to the data collected was obtained from all the patients, and the study was approved by our Institutional Review Board.

German-ENSAT cohort. To confirm the results, an independent cohort derived from the German ACC Registry group was analyzed. As recently described, 130 ACC tumors samples from primary surgery were collected from patients undergoing adrenalectomy between 1989 and 2007 and assembled into 3 tissue microarrays (TMA; refs 25, 26). Characteristics and inclusion criteria of the entire cohort have previously been described in detail (27). Follow-up information was provided at the time of any relevant change in the course of the disease or at least every 6 months. The German ACC Registry is approved by the Ethics Committee of the University of Wuerzburg, Germany, and all patients provided written informed consent.

Pathologic examination and immunohistochemical staining

For diagnosis and scoring, the tumors were fixed in formalin, embedded in paraffin, and 4-μm sections were cut and stained with hematoxylin–eosin–saffron. The Weiss score was established on the basis of 9 Weiss histologic criteria (20, 21). Immunohistochemistry for β-catenin (BD Bioscience; 1/400) was performed as previously described (11, 12). Briefly, for the Cochin-COMETE cohort, sections, 4-μm thick, from formalin-fixed tissue embedded in paraffin were mounted on Superfrost Plus glass slides. For the German-ENSAT cohort, TMAs were prepared from formalin-fixed paraffin-embedded tissue specimens using a manual device. Five representative tissue scores per patient were arrayed to obtain a representative pattern of the tumor. Immunostaining was assessed by a single pathologist experienced in β-catenin immunohistochemistry reading (F.T.), blinded to mutational status and clinical information. Nuclear staining was quantitatively assessed based on 1,000 nuclei, using the Leica QWinV3 software (Leica Microsystems).

Nucleic acid extraction and mutation analysis of *CTNNB1* and *APC*

For the 49 ACC from the Cochin-COMETE cohort with frozen tissue available, DNA was extracted and purified by cesium chloride gradient ultracentrifugation as previously reported (28). For *CTNNB1* mutation analysis, exon 3 and the flanking 5′ intronic sequences were amplified by PCR and sequenced as previously described (11, 12). Nucleotides were numbered in accordance with the reference sequence for *CTNNB1*, that is, β-catenin gene (GenBank accession no. NM_001904; Supplementary Data 1). In patients without *CTNNB1* mutation, *APC* was sequenced on its hotspot, that is, mutation cluster region (Supplementary Data 1). To perform this procedure, both strands of the amplified products were directly sequenced on an automated sequencer (ABI 3700; PerkinElmer). Nucleotides were numbered in accordance with the reference sequence

for *APC* (GenBank accession no. NM_001127510). Additionally, in a patient with familial adenomatous polyposis and ACC, denaturing high performance liquid chromatography (DHPLC) was used for mutation prescreening of the coding sequences of *APC*. The DHPLC was optimized for a rapid screening of the 15 exons and the splice junctions of the *APC* gene. The abnormal profiles detected by the DHPLC were subsequently sequenced.

Statistical analysis

Data are reported as means (interquartile) or percentages, as appropriate. Because of non-normal distribution of several variables and the small number of subjects, nonparametric statistical methods were used to analyze the relationships between variables when appropriate (Fisher exact, Kruskall–Wallis, or Wilcoxon tests). For the survival analysis, the patients' follow-up was censored if the patient was still alive at last follow-up. Survival was estimated by the method of Kaplan–Meier and the log-rank test was used to compare survival curves. The Hazard ratio (HR) and its 95% confidence interval (CI) were estimated using Cox's proportional hazards regression model in a uni- and multivariate analysis. Disease-free survival was only analyzed in patients with complete surgical resection and was defined as the time from the date of tumor resection to the first evidence of relapse or last follow-up without evidence for disease. To study the independent effect of β-catenin on survival, the multivariate models were adjusted for β-catenin nuclear staining (presence/absence), and for main known clinical prognostic variables that had $P < 0.15$ in univariate analyses, that is, age (by 10 year increments), ENSAT stage (3 or 4 versus 1 or 2) and glucocorticoid secretion (yes versus no), as previously published by our group (24). The variables included in the final model were first determined on the Cochin-COMETE cohort data set, then secondarily applied to the German-ENSAT cohort, in 2 separate analyzes. All tests were 2-sided. For all tests, statistical significance was defined by $P < 0.05$. Statistical analysis was carried out with the SAS package Version 9.2 (SAS Institute Inc.) by an experienced statistician (S.Gr.).

Results

Cochin-COMETE cohort

Patients. Seventy-nine patients with resected primary ACC (23 males and 56 females; median age: 43.8 years; interquartile: 30.7–58.6) constitute the Cochin-COMETE cohort population. The main patients' characteristics are detailed in Table 1. Tumors were mainly diagnosed on endocrine features (49.4%, $n = 39$), mass syndrome (22.8%, $n = 18$), or as incidentaloma (19%, $n = 15$), and were right sided (41.8%, $n = 33$). Secretory activity was demonstrated in 74.7% of patients ($n = 59$), with glucocorticoid secretion (alone or in association with other secretion) in 64.6% of patients ($n = 51$). The median tumor size was 11 cm (interquartile: 8–15) and the median tumor weight was 314 g (interquartile: 135–920). Overall, 6.3% ($n = 5$), 44.3% ($n = 35$), 12.7% ($n = 10$), and 36.7% ($n = 29$) of the patients were classified as ENSAT stages I, II, III, and IV, respectively.

Pathologic and mutational characteristics of the ACC. Pathologic and mutational characteristics of the ACC are presented in Table 2. The mitotic count was over 5 mitoses by 50 high-power fields in 54.4% ($n = 43$), and over 20 in 22.8% ($n = 18$). A β-catenin nuclear staining was present in 39.2% of the ACC ($n = 31$; Fig. 1A and B) and β-catenin mutations were observed in 16.3% (8 of the 49 tumors with available frozen tissue). In addition, 1 patient had an *APC* mutation (within a familial adenomatous polyposis), without any *CTNNB1* mutation. Mutations were overall observed in 18.4% (9 of 49) of the cohort. The details of the mutations are given in Table 3.

Baseline characteristics of the patients and pathologic characteristics of the ACC according to β-catenin status. Baseline characteristics of patients and pathologic characteristics of the tumors according to β-catenin nuclear staining are, respectively, presented in Tables 1 and 2. β-Catenin nuclear staining was present in 39.2% (31 of 79) of the population. The presence of β-catenin nuclear staining was significantly associated with ENSAT stages III and IV, that is, the presence of locally advanced and metastatic disease ($P = 0.01$). The presence of β-catenin nuclear staining was significantly associated with the presence of tumor necrosis ($P = 0.03$), a high mitotic count (>5 and >20 mitoses by 50 high-power fields $P < 0.0001$), and *CTNNB1* and/or *APC* mutations ($P < 0.0001$), as shown in Table 2. No difference was observed among the baseline characteristics of the patients according to the presence or absence of mutation (data not shown). The presence of *CTNNB1*/*APC* mutations was only significantly associated with a higher mitotic count ($P = 0.009$).

Overall and disease-free survival of the French-COMETE cohort and correlation with β-catenin nuclear staining and CTNNB1/APC mutations. Follow-up information was available for all patients. The median follow-up since surgery, calculated according to the inverse Kaplan–Meier method, was 29 months.

The median overall survival for the entire cohort was 54.5 months. Patients with ACC without β-catenin nuclear staining survived significantly longer than patients with the presence of β-catenin nuclear staining (median 110 months versus 23 months, respectively, log-rank $P = 0.001$; Fig. 2A; see Supplementary Data 2 for details regarding survival).

For the 49 patients for whom the *CTNNB1* and *APC* mutations were documented, the survival analysis showed that absence of *CTNNB1* or *APC* mutations was also associated with a longer survival (median 58 months versus 24 months, respectively, log rank $P = 0.039$; data not shown).

The results of the univariate and multivariate Cox proportional hazards models are shown in Table 4. After adjustment for age at diagnosis, ENSAT stage and glucocorticoid secretion (previously reported by our group to be independent predictive factors of poor prognosis; ref 24), the presence of β-catenin nuclear staining remained an

Table 1. Baseline characteristics of the patients according to the presence of a β-catenin nuclear staining in the Cochin-COMETE cohort

		β-Catenin nuclear staining		
	Overall ($n = 79$)	**Absent ($n = 48$)**	**Positive ($n = 31$)**	***P***
Age, y				
Median (interquartile)	44 (30.7–58.6)	43 (32–57.5)	44 (31–59)	0.85
Sex				
Female	56 (70.9)	31 (65.3)	25 (80.6)	0.14
Circumstance of diagnosis				
Endocrine features	39 (49.4)	20 (40.8)	19 (61.3)	0.25
Mass syndrome	18 (22.8)	12 (24.5)	6 (19.3)	
Incidentaloma	15 (19)	12 (24.5)	3 (9.7)	
Others	7 (8.9)	4 (8.2)	3 (9.7)	
Side				
Right	33 (41.8)	22 (45.8)	11 (35.5)	0.48
Functional status of ACC				
No secreting tumor	20 (25.3)	16 (33.3)	4 (19.9)	0.06
Secreting tumor	59 (74.7)	32 (66.7)	27 (87.1)	
No glucocorticoid secretion	28 (35.4)	21 (43.7)	7 (22.6)	0.09
Glucocorticoid secretion	51 (64.6)	27 (56.3)	24 (77.4)	
Tumor size, cm				
Median (interquartile)	11 (8–15)	10 (8–15)	12 (8.2–20)	0.09
Tumor weight, g				
Median (interquartile)	314 (135–920)	230 (104—1,000)	419 (228–920)	0.09
ENSAT Tumor stage				
I	5 (6.3)	3 (6.3)	2 (6.5)	0.01[a]
II	35 (44.3)	27 (56.2)	8 (25.8)	
III	10 (12.7)	6 (12.50)	4 (12.9)	
IV	29 (36.7)	12 (25)	17 (54.8)	

NOTE: All values are given in *n* (%), unless otherwise mentioned.
[a]I , II vs III, IV.

independent predictive factor of survival (adjusted HR 2.3; 95% CI, 1.2–4.5).

Disease-free survival was only estimated for patients who underwent complete surgical resection ($n = 50$). The median disease-free survival for the entire cohort was 142 months. As reported for overall survival, disease-free survival was statistically better for patients for whom β-catenin nuclear staining was absent (at 60 months 72.3% versus 34.6%, respectively, log-rank $P = 0.019$; Fig. 2A) and when *CTNNB1* and *APC* mutations were absent (median 58 months versus 24 months, respectively, log-rank $P = 0.006$; data not shown). In multivariate analysis including the same previous parameters, all of them [i.e., age at diagnosis (by 10 years), ENSAT stage, glucocorticoid secretion, and β-catenin nuclear staining] were found to be independent predictive factors of disease-free survival (data not shown).

German-ENSAT cohort

Ninety-two tumor samples of TMAs were available with which to quantify β-catenin nuclear staining by immunohistochemistry (Fig. 1C and D). Median follow-up for surviving patients was greater than 50 months.

Compared to the Cochin-COMETE cohort, the patients with ACC had similar demographic (age, sex) and tumor characteristics (laterality, circumstance of diagnosis, secretion, tumor size, and weight), with the exception that there were more ENSAT stage III—IV tumors in the German-ENSAT cohort (57.6% versus 49.4%, $P = 0.01$), higher Weiss scores (7–9) in the Cochin-COMETE cohort (39.2% versus 16.3%, $P < 0.0001$), and a more frequent mitotic count of more than 5 mitoses per 50 high-power fields in the German-ENSAT Cohort (73.9% versus 54.4%, $P = 0.01$). β-Catenin nuclear staining was present in 33 ACC (35.9%) in the ENSAT cohort, a proportion comparable to those observed in the Cochin-COMETE cohort (39.2%, $P = 0.75$).

Median overall survival in the German-ENSAT cohort was 43 months and the 1-, 2-, and 5-year survival rates were 77.3% (67–84.7), 60.2% (49.2–69.6), and 43% (32–53.5), respectively.

The results of the German-ENSAT cohort confirmed the results of the Cochin-COMETE cohort showing a shorter

Table 2. Pathologic characteristics of the ACC according to the presence of a β-catenin nuclear staining in the Cochin-COMETE cohort

		β-Catenin nuclear staining		
	Overall ($n = 79$)	**Absent ($n = 48$)**	**Present ($n = 31$)**	***P***
High nuclear grade				
Present	72 (91.1)	43 (89.6)	29 (93.5)	0.69
Mitotic count				
Median (interquartile)	6 (1–16)	2 (0–8.5)	16 (6–34)	<0.0001
>5	43 (54.4)	18 (37.5)	25 (80.7)	0.0002
>20	18 (22.8)	4 (8.3)	14 (45.2)	0.0002
Atypical mitosis				
Present	23 (29.1)	10 (20.8)	13 (41.9)	0.07
<25% clear cells				
Present	76 (96.2)	46 (95.9)	30 (97.8)	1
Diffuse architecture				
>33% surface				
Present	75 (94.9)	45 (93.8)	30 (96.8)	1
Necrosis				
Present	64 (81)	35 (72.9)	29 (93.6)	0.03
Sinusoid invasion				
Present	27 (34.2)	14 (29.2)	13 (48.2)	0.32
Venous invasion				
Present	36 (45.6)	18 (37.5)	18 (58.1)	0.1
Capsular invasion				
Present	40 (50.6)	21 (43.8)	19 (61.3)	0.16
CTNNB1 or *APC* mutations[a]				
Present	9/49 (18.4)	0/27 (0)	9/22 (40.9)	<0.0001

NOTE: All values are given in *n* (%), unless otherwise mentioned.
[a]Data available for only 49 sequenced tumors.

overall survival in ACC patients with β-catenin nuclear staining than in those without β-catenin nuclear staining (median 21 months versus 62 months, respectively, log-rank $P = 0.01$) and a shorter disease-free survival (median 9 months versus 34 months, respectively, log-rank $P = 0.009$; Fig. 2C and D). After adjustment for age at diagnosis, ENSAT stage, and glucocorticoid secretion, the presence of β-catenin nuclear staining remained an independent predictive factor of survival (adjusted HR 2.4; 95% CI, 1.3–4.6; Table 4).

Discussion

The Wnt/β-catenin signaling pathway has been identified as one of the key signaling pathways in cancers (14–17), having an essential pathogenic role in regulating cell growth, motility, and differentiation (29). Increasing clinical and scientific evidence supports the importance of the activation of this pathway in adrenal cortex tumorigenesis (11–13), as previously shown in a variety of human cancers (14–17). In this study, we used β-catenin immunohistochemistry and *CTNNB1/APC* mutations analysis to assess the activation of the Wnt/β-catenin signaling pathway and to investigate its prognostic value in a series of 79 patients with primary resected ACC. Our results show that Wnt/β-catenin signaling pathway activation, evidenced by the presence of β-catenin nuclear staining, is an independent prognostic factor of overall and disease-free survival in patients with resected primary ACC. The confirmation of these results in a second independent cohort supports and highlights the robustness of this finding. This study suggests the possible use of β-catenin immunohistochemistry as a reliable clinical tool and underlines that the study of the Wnt/β-catenin signaling pathway is a promising research field for better understanding and management of adrenocortical tumors.

Prognostic tools are essential in oncologic care. In addition to the ENSAT classification based on morphologic features, there is a growing interest in prognostic pathologic or molecular markers on the basis of tumor biology such as mitotic index (8, 20, 30), Ki-67 labeling index (31), steroidogenic factor 1 (SF-1) labeling (32), IGF-II overexpression (33), LOH in 17p13 (34), TP53 mutations (35), or gene expression profiling (9, 10). The activation of the Wnt/β-catenin signaling pathway has previously been suggested to be associated with a more aggressive phenotype in

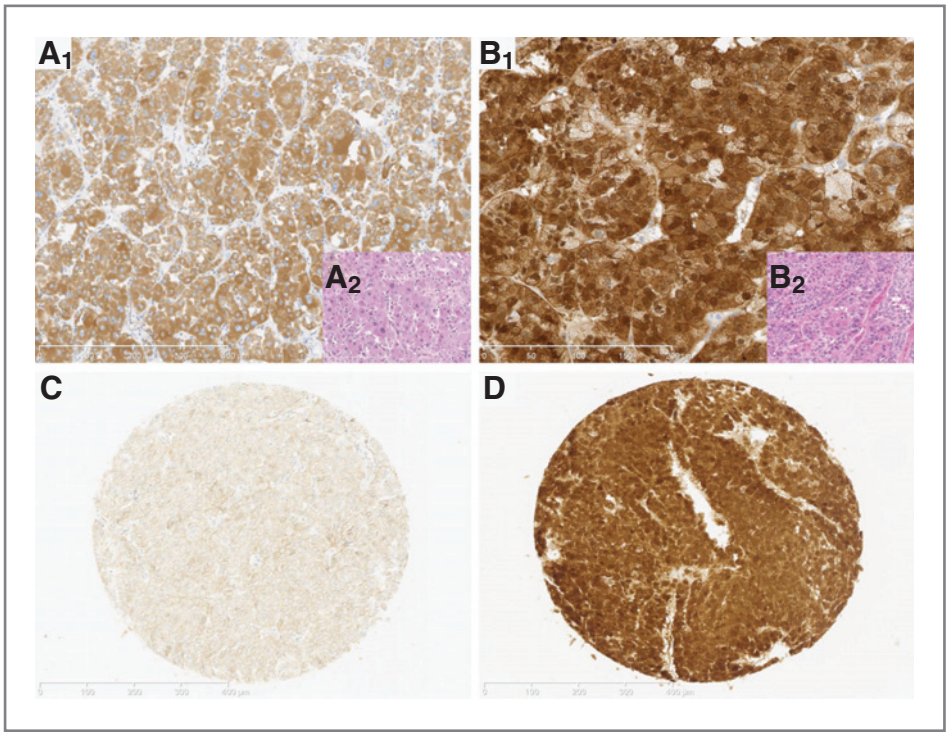

Figure 1. β-catenin immunohistochemistry and hematoxylin–eosin–saffron staining of ACC. A, ACC showing only β-catenin cytoplasmic accumulation (A_1, ×100) and its hematoxylin—eosin–saffron (A_2, ×100). B, ACC showing β-catenin cytoplasmic and nuclear accumulation (B_1, ×200), and its hematoxylin-eosin-saffron (B_2, ×100). C, ACC in TMA showing no β-catenin accumulation but only membranous labeling (×100). D, ACC in TMA showing β-catenin cytoplasmic and nuclear accumulation (×100).

PPNAD and ACC (12). Our results confirm for the first time that Wnt/β-catenin signaling pathway activation, demonstrated by the presence of nuclear staining in ACC, might lead to a more aggressive tumorigenesis and can also serve as a prognostic factor. Indeed, we observed that locally advanced stage (stage III) and metastatic ACC (stage IV) were more frequent and overall and disease-free survival statistically decreased when β-catenin nuclear staining was present. Interestingly, the presence of β-catenin nuclear staining was associated with increased mitotic count. This is in accordance with the well-known function of Wnt/β-catenin signaling pathway regulating cell growth, motility, and differentiation (29) and could explain in part the worse prognosis of ACC with activation of the Wnt/β-catenin signaling pathway. Our observation is consistent with other observations in several models including liver (36), colonic (37), or thyroid (38) carcinomas, indicating that β-catenin accumulation is associated with aggressive behavior in these types of tumors. Nevertheless, the available literature showed mixed results regarding the relationship between β-catenin staining and outcome in various other kinds of tumors such as breast cancer (39) or medulloblastoma (40), where nuclear β-catenin accumulation appears to be a marker of favorable outcome. This could be due to a tissue specificity of the target genes of the Wnt/β-catenin signaling pathway, and further investigations are necessary to identify relevant target genes of the Wnt/β-catenin signaling pathway in adrenal cortex and adrenocortical tumors. Moreover, as recently reported (32), SF-1 expression assessed by immunohistochemistry is also a stage-independent prognostic marker in ACC. Regarding its additional value to β-catenin nuclear staining in the German-ENSAT Cohort, SF-1 has a prognostic significance only on overall survival in patients with positive nuclear β-catenin staining, but not in patients with negative nuclear β-catenin staining, or on disease-free survival of R0 resected patients (data not shown).

A discrepancy between *CTNNB1*/*APC* mutations and β-catenin nuclear staining was shown. Indeed, among the 49 ACC sequenced, 13 (26.5%) present a β-catenin nuclear staining by immunohistochemistry without any detected mutation. These results confirm our previous

Table 3. Description of *CTNNB1* and *APC* mutations in the Cochin-COMETE cohort ($n = 49$ patients)

Type of mutation	Number of cases
CTNNB1 mutations	
c. /p.Tyr30X heterozygous	1
c. /p.Ile35 heterozygous	1
c. /p.Thr41 heterozygous	1
c. /p.Ser45 heterozygous	3
c. /p. Ser45del heterozygous	1
c. 26993 del 489pb heterozygous	1
APC mutations	
Somatic: c.3234T>A / p.Tyr1078X heterozygous	1
Germline: c.4689_4690ins4 / p.Leu1563FsX1567 heterozygous	

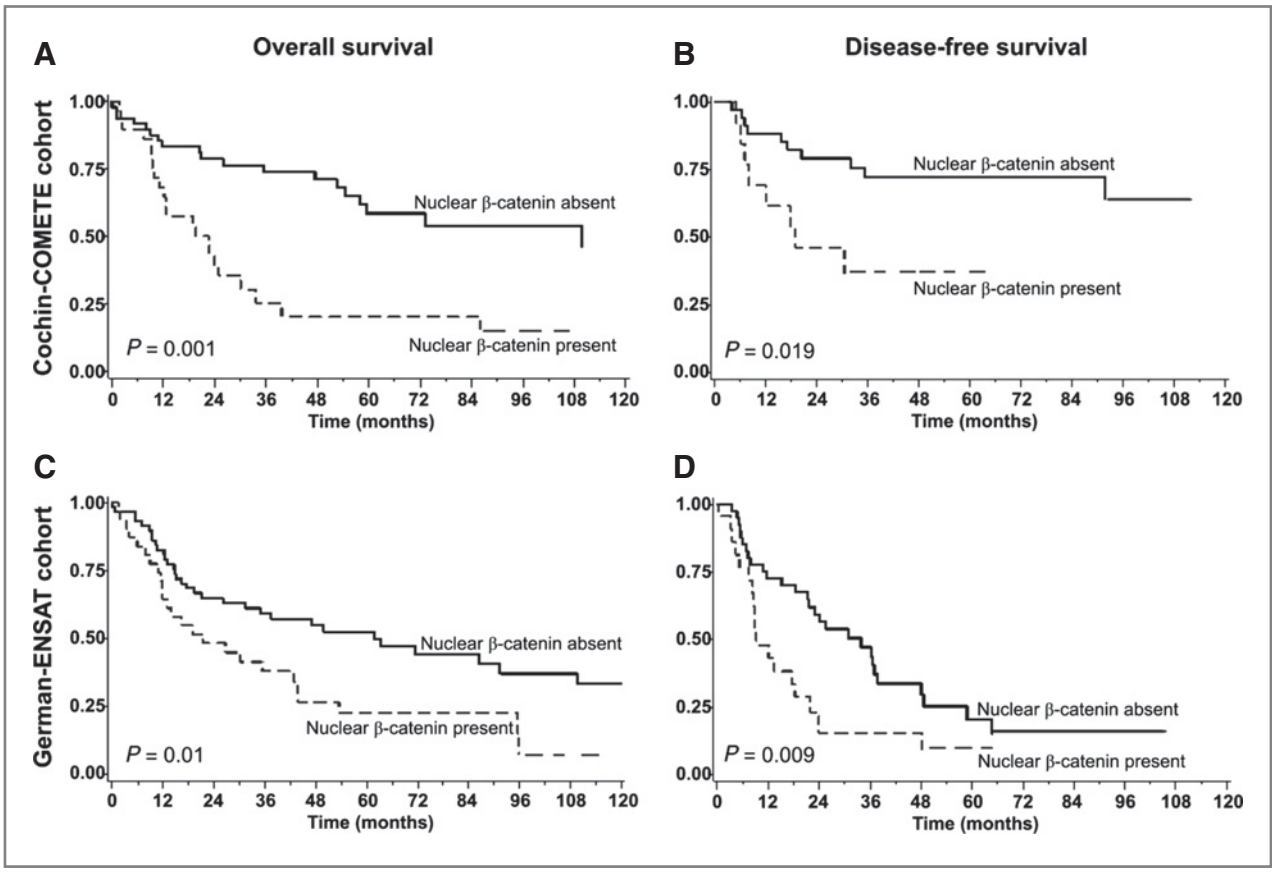

Figure 2. Patients' overall and disease-free survival according to the presence of β-catenin nuclear staining (Kaplan–Meier method). A, overall survival of the patients ($n = 79$) of the Cochin-COMETE cohort according to the presence (dotted line) or absence (continuous line) of β-catenin nuclear staining. B, disease-free survival of the patients with complete surgical resection ($n = 50$) of the Cochin-COMETE cohort according to the presence (dotted line) or absence (continuous line) of β-catenin nuclear staining. C, overall survival of the patients ($n = 92$) of the German-ENSAT cohort according to the presence (dotted line) or absence (continuous line) of β-catenin nuclear staining. D, disease-free survival of the patients with complete surgical resection ($n = 67$) of the German-ENSAT cohort according the presence (dotted line) of absence (continuous line) of β-catenin nuclear staining.

study of a smaller series of 13 ACC (11) and suggest the involvement of other mechanisms in Wnt/β-catenin signaling pathway activation, such as a cross talk or mutations in other genes that remain to be identified. It is likely that all ACC with β-catenin nuclear staining present an activation of the Wnt/β-catenin signaling pathway. From this point of view, the ACC with such an activation explained by another mechanism than a *CTNNB1* mutation are likely to behave clinically like the ACC harboring a *CTNNB1* mutation.

CTNNB1 mutations have been demonstrated to initiate benign tumor development, as well as to promote progression towards malignancy in adrenocortical tumor (18). The question of whether it is an early or late event in adrenocortical tumorigenesis is still controversial. An argument for a 2-step model for β-catenin–induced tumorigenesis is that *CTNNB1* mutations are recorded both in benign and malignant adrenocortical tumor and that transgenic mice with constitutive activation of the Wnt/β-catenin signaling pathway in the adrenal cortex showed a range of abnormal adrenal phenotypes (18), suggesting the need for additional events.

Our results provide an argument for the implication of the Wnt/β-catenin signaling pathway in the progression of ACC. Reasons why Wnt/β-catenin signaling pathway activation is associated with such a phenotype remain unknown and further studies are needed to identify tissue-specific downstream target genes of this pathway. However, drugs targeting β-catenin–dependent transcriptional activity have already shown efficiency on adrenocortical cell models (41), and consequently the Wnt/β-catenin signaling pathway could be attractive for developing novel targeted therapies for ACC, a disease for which systemic available treatments lack efficiency.

Conclusion

Previous studies have reported the importance of the Wnt/β-catenin signaling pathway activation in adrenal cortex tumorigenesis, but for the first time we correlate, using 2 independent cohorts, the activation of this pathway with clinical characteristics, pathologic features, and outcome. In patients whose tumors present β-catenin nuclear

Table 4. Univariate and multivariate Cox regression analyses of risk of death in the Cochin-COMETE and the German-ENSAT cohort

			Univariate analysis		Multivariate analysis	
		n	HR [95%CI]	*P*	HR [95%CI]	*P*
Cochin-COMETE cohort ($n = 79$ patients)						
Age at diagnosis (by 10 y)		79	1.2 [1.0–1.5]	0.11	1.2 [1.0–1.5]	0.04
ENSAT stage	1–2	40	1 (reference)		1 (reference)	
	3	10	3.6 [1.4–9.6]	0.0090	3.4 [1.3–9.2]	0.01
	4	29	6.7 [3.2– 14.2]	<0.0001	5.1 [2.3–11.3]	<0.0001
Glucocorticoid secretion	Absent	28	1 (reference)	0.0028	1 (reference)	0.17
	Present	51	3.3 [1.5–7.5]		1.8 [0.8– 2]	
β-catenin nuclear staining	Absent	48	1 (reference)	0.0003	1 (reference)	0.016
	Present	31	3.3 [1.7–6.2]		2.3 [1.2–4.5]	
German-ENSAT cohort ($n = 92$ patients)						
Age at diagnosis (by 10 y)		92	1.0 [0.8–1.2]	0.8908	1.1 [0.9–1.3]	0.2658
ENSAT score	1-2	39	1 (reference)		1 (reference)	
	3	29	1.6 [0.9–3.2]	0.1368	1.8 [0.9–3.5]	0.0813
	4	24	4.5 [2.3–8.7]	<0.0001	5.3 [2.7–10.6]	<0.0001
Glucocorticoid secretion	Absent	29	1 (reference)		1 (reference)	
	Present	36	0.7 [0.4–1.3]	0.2441	0.7 [0.4–1.2]	0.2046
	Missing[a]	27	0.8 [0.4–1.6]	0.6174	0.8 [0.4–1.7]	0.6050
β-Catenin nuclear staining	Absent	59	1 (reference)		1 (reference)	
	Present	33	1.9 [1.1–3.2]	0.0180	2.4 [1.3–4.6]	0.0051

[a]Missing data.

staining, overall and disease-free survival were significant shorter. These results highlight the importance of this pathway for a better understanding of ACC pathogenesis and its potential implication in the management of ACC, as a prognostic factor or a future therapeutic target.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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