Mutations of *β-Catenin* in Adrenocortical Tumors: Activation of the Wnt Signaling Pathway Is a Frequent Event in both Benign and Malignant Adrenocortical Tumors

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Abstract

Adrenocortical cancer is a rare cancer with a very poor prognosis. The genetic alterations identified to date in adrenocortical tumors are limited. Activating mutations of the Wnt signaling pathway have been observed in more frequent cancers, particularly digestive tract tumors. We investigated whether Wnt pathway activation is involved in adrenocortical tumorigenesis. In a series of 39 adrenocortical tumors, immunohistochemistry revealed abnormal cytoplasmic and/or nuclear accumulation of β-catenin in 10 of 26 adrenocortical adenomas and in 11 of 13 adrenocortical carcinomas. An activating somatic mutation of the *β-catenin* gene was shown in 7 of 26 adrenocortical adenomas and in 4 of 13 adrenocortical carcinomas; these mutations were observed only in adrenocortical tumors with abnormal β-catenin accumulation and most were point mutations altering the Ser^{45} of exon 3 (in the consensus GSK3-β/CK1 phosphorylation site). Functional studies showed that the activating Ser^{45} *β-catenin* mutation found in the adrenocortical cancer H295R cell line leads to constitutive activation of T-cell factor–dependent transcription. This is the first molecular defect to be reported with the same prevalence in both benign (27%) and malignant (31%) adrenocortical tumors. *β-Catenin* mutations are also the most frequent genetic defect currently known in adrenocortical adenomas. In adrenocortical adenomas, β-catenin alterations are more frequent in nonfunctioning tumors, suggesting that β-catenin pathway activation might be mostly involved in the development of nonsecreting adrenocortical adenomas and adrenocortical carcinomas. The very frequent and substantial accumulation of β-catenin in adrenocortical carcinomas suggests that other alterations might also be involved. This finding may contribute to new therapeutic approaches targeting the Wnt pathway in malignant adrenocortical tumors, for which limited medical therapy is available. (Cancer Res 2005; 65(17): 7622-7)

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Introduction

Adrenocortical tumors can be diagnosed as adrenocortical adenomas or adrenocortical carcinomas, which have a very poor prognosis (1–3). Most sporadic adrenal tumors are monoclonal, suggesting that a somatic genetic defect occurs early during tumorigenesis (4). Somatic mutations of the tumor suppressor gene *TP53* were initially reported, mostly in adrenal carcinomas (5). More recently, we have reported somatic inactivating mutations of the tumor suppressor gene *PRKAR1A* in a subgroup of adrenocortical adenomas responsible for Cushing's syndrome (6). Consistent with the occurrence of adrenocortical tumors in the Li-Fraumeni and Beckwith-Wiedemann syndromes (7), allelic losses [loss of heterozygosity (LOH)] at the *TP53* locus at 17p13 and the insulin-like growth factor II (*IGF-II*) locus at 11p15 have been observed in adrenocortical carcinomas (8). IGF-II overexpression is a major characteristic of adrenocortical carcinomas and has been suggested as a prognostic factor (9). However, the physiopathology of adrenocortical tumors is not well documented because very few genetic alterations have been identified in these tumors (10). Molecular studies have pinpointed activating mutations of the Wnt signaling pathway as the cause of many cancers (11–13). β-Catenin plays a central role in the Wnt 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) in the Wnt signaling pathway. In the absence of Wnt signaling, the level of β-catenin is low: β-catenin is phosphorylated at critical NH_2-terminal residues by the GSK3-β bound to a scaffolding complex of axin and adenomatous polyposis proteins (APC) and subsequently the phosphorylated protein is degraded by the ubiquitin-proteasome system (14). Wnt stimulation leads to the inactivation of GSK3-β and thereby the stabilization of β-catenin in the cytoplasm. Consequently, β-catenin becomes available to bind the TCF/LEF family of transcription factors and to induce target gene expression (15). We report a study of the status of β-catenin by immunohistochemistry, DNA sequencing, and functional study of the H295R cell line to assess its contribution to adrenocortical tumorigenesis.

Materials and Methods

Patients. Patients included in the study were investigated for a sporadic adrenocortical tumor (other than Conn's adenoma) in the Endocrinology Department of Cochin Hospital. None of the 39 patients presented features of any adrenocortical tumor–predisposing syndrome

(Beckwith-Wiedemann, Carney complex, McCune-Albright, Multiple Endocrine Neoplasia type 1, or Li-Fraumeni syndrome). Clinical data, hormonal status, and tumor stage (McFarlane classification) were assessed as previously described (16, 17). Informed consent for the analysis of leukocyte and tumor DNA and for access to the data collected was obtained from all the patients, and the study was approved by an institutional review board (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Cochin Hospital, Paris). The allelic status at the *11p15* and *17p13* loci and IGF-II messenger abundance in tumors were determined as previously described (8). After surgery, patients were examined twice a year for 2 years and annually thereafter. Hormonal evaluation, chest X-ray, and computerized tomography scans of the abdomen and thorax were carried out at each evaluation for adrenocortical carcinoma. The patients were followed until their date of death, their last examination, or the end of the follow-up period. The minimal and the maximal follow-up periods were 12 and 159 months, respectively (mean 81.9 ± 32.3).

Human H295R cell line. The human adrenocortical cancer H295R cell line was grown as previously described (18) in DMEM/Ham's F-12 supplemented with 2% Ultroser G (Biosepra, Fremont, CA), 2 mmol/L glutamine, 5 μg/mL insulin, 5 μg/mL transferrin, 5 ng/mL selenium, 50 units/mL penicillin, and 50 mg/mL streptomycin.

Microscopy. The tumors were fixed in formalin, embedded in paraffin, and 4 μm sections were cut and stained with H&E-safran. The sections were examined to assess their Weiss score (0 to 9) on the basis of the presence or absence of the following nine histologic features: high mitotic rate, atypical mitoses, high nuclear grade, low percentage of clear cells, necrosis, diffuse tumor architecture, capsular invasion, sinusoidal invasion, and venous invasion (19).

Immunohistochemical staining. Sections, 4 μm thick, from formalin-fixed tissue embedded in paraffin were mounted on Superfrost/Plus glass slides. Immunohistochemistry for β-catenin was done as previously described (15). For negative controls, some sections were incubated without the primary antibody. The slides were counterstained with Mayer hematoxylin. Immunostaining was assessed by an investigator blinded to McFarlane stage, Weiss score, and outcome. The entirety of β-catenin–stained sections was examined. Immunohistochemical labeling was evaluated for the presence of membranous, cytoplasmic, and nuclear staining by a qualitative assessment, and cytoplasmic and/or nuclear staining was recorded as intracellular accumulation. The intensity of staining was not scored.

Nucleic acid extraction and mutation analysis of the *β-catenin* gene. DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification KIT (Promega Corp., Madison, WI). Tumor DNA and RNA were purified by cesium chloride gradient ultracentrifugation as previously reported (20). cDNA was synthesized by Moloney murine leukemia virus-reverse transcriptase (Invitrogen, Groningen, the Netherlands) using 1 μg of total RNA in a final volume of 40 mL. For mutation analysis, exon 3 and the flanking intronic sequences of the *β-catenin* gene were amplified by PCR from both tumoral and leukocyte DNA. The primers used were βCATEX2F (GAAAATCCAGCGTGGACAATG) and βCATEX4R (TCGAGTCATTGCATACTGTCC). Both strands of the amplified products were directly sequenced on an automated sequencer (ABI 3700; Perkin-Elmer, Boston, MA). To search for large *β-catenin* gene deletions, exon 3 was also amplified from tumor cDNA using the primers βCATF1 (GCGTGGACAATGGCTACTCAAG) and βCATR2 (TTCAGCACTCTGCTTGTGGTCC). The primer βCATR1 (TTCAGGGATTGCACGTGTGGC) was also used for sequencing for large *β-catenin* gene deletion analysis. Mutations were confirmed twice on two independent experiments.

Cell line, transfection, and reporter assays. The human adrenocortical cancer H295R cell line was maintained as previously described (18).

Transient transfections were done when cells were 60% to 70% confluent in 12-well plates using Effectene transfection reagent (Qiagen GmbH, Hilden, Germany). The total amount of transfected DNA (1.25 μg per well) was kept constant by adding pcDNA3. A TK-Renilla plasmid (10 ng) was included in each transfection for monitoring of transfection efficiency. PTOPFLASH [containing two copies of the β-catenin/T-cell factor (TCF)-binding sites], pFOPFLASH (containing two mutated copies of the β-catenin/TCF-binding sites), and the expression plasmid encoding the dominant-negative form of TCF (pΔNTCF4) were kindly provided by H. Clevers (Utrecht, the Netherlands); 0.25 μg of pTOPFLASH or pFOPFLASH plasmid and growing quantities of pΔNTCF4 (from 50 ng to 1 μg) were added per well. All experiments were done in triplicate and repeated at least thrice. Cells were lysed 36 hours after transfection and the luciferase and renilla activities were assayed using Promega Dual Luciferase Reporter Assay (Promega). The activity of the reporter constructs was expressed as normalized relative light units.

Statistical analysis. The χ^2 test was used to compare rates of β-catenin delocalization or mutations between tumor groups. Statistical analyses were done using the StatView 5.0 program (SAS Institute, Cary, NC); significance was set at $P < 0.05$.

Results

Clinicopathologic results. The clinicopathologic results are summarized in Table 1. The 39 patients (34 females) were 25 to 79 years old (mean ± SD: 45.9 ± 15.1). Thirty-two patients (82%) presented an adrenal steroid excess (mainly cortisol and/or androgens). Thirty-two patients (82%) underwent curative surgery. Thirty patients (77%) had localized tumors (McFarlane stage I and II). Nine patients (23%) had disseminated tumors at diagnosis (McFarlane stage IV); four patients had tumors invading adjacent organs and five patients had distant metastases. All of the nine patients with obviously malignant tumors (stage IV) had a Weiss score of ≥3 and 26 patients (87%) with localized tumors (stage I and II) had a Weiss score of ≤2.

β-Catenin expression. A hallmark of active β-catenin signaling in the tumor is a cytoplasmic/nuclear staining for β-catenin (13). We used immunohistochemistry to examine the cellular localization of β-catenin in a cohort of 39 adrenocortical tumors and in human H295R adrenocortical cancer cell lines (Table 2). Cytoplasmic/nuclear staining was observed in 10 of the 26 adrenocortical adenomas (38%), but β-catenin accumulation was focal and present in only a few tumor cells (Fig. 1). Interestingly, most adrenocortical adenomas showing an abnormal β-catenin immunostaining were nonfunctioning adrenocortical tumors (i.e., not responsible for steroid oversecretion; Table 1): 5 of 6 (83%) of the nonfunctioning adrenocortical adenomas showed an abnormal β-catenin immunostaining, in contrast to 5 of 20 (25%) of the secreting adrenocortical adenomas (responsible for glucocorticoid oversecretion; $P < 0.01$, χ^2 test).

Eleven of the 13 adrenocortical carcinomas (77%) had a diffuse cytoplasmic/nuclear β-catenin accumulation (Fig. 1). Thus, an abnormal cytoplasmic/nuclear localization of β-catenin is frequent in adrenocortical tumor. However, the pattern differs between tumors. The abnormal β-catenin immunostaining was focal in most adrenocortical adenomas and diffuse in adrenocortical carcinomas. Interestingly, the same abnormal nuclear and cytoplasmic β-catenin localization was also observed in H295R cells (Fig. 1).

***β-Catenin* genetic analysis.** The results of *β-catenin* genetic analysis in all the tumors and the H295R cells are summarized in Table 2. A molecular alteration of the *β-catenin* gene was observed in 11 of 39 adrenocortical tumors (28%) and in H295R cells. Most of these mutations (8 of 12) were point mutation altering the Ser^{45} of exon 3 and resulting in an amino acid substitution. Deletion or insertion leading to alteration of exon 3 was observed in the other tumors. None of these genetic alterations were observed in leukocyte DNA of the patients, demonstrating their somatic origin.

Genetic alterations were observed both in adrenocortical adenomas and adrenocortical carcinomas. They were observed only in tumors showing an abnormal β-catenin immunostaining pattern. Among the nonfunctioning adrenocortical adenomas, 4 of 6 (67%) harbor a somatic *β-catenin* mutation, in contrast to 3 of 20 (15%) of the

Table 1. Clinicopathologic and genetic features [*11p15* uniparental disomy, *17p13* allelic loss (LOH), and overexpression of the *IGF-II* gene] in 39 patients with sporadic adrenocortical tumors

Patients	Age (y)/sex	Functional status	Tumor weight (g)	McFarlane staging	Weiss score	*11p15*	Genetic abnormalities *17p13*	IGF-II	Curative surgery	Clinical status at last follow-up
1 (AA)	28/F	GC	19.0	I	0	0	0	0	Yes	CR
2 (AA)	28/M	GC	28.0	I	0	0	0	NI	Yes	CR
3 (AA)	71/F	NF	32.5	I	0	0	0	0	Yes	CR
4 (AA)	61/F	NF	15.0	I	0	0	0	0	Yes	CR
5 (AA)	44/F	GC	11.1	I	0	NI	0	0	Yes	CR
6 (AA)	34/F	GC	13.9	I	0	0	0	0	Yes	CR
7 (AA)	28/F	GC	17.0	I	0	0	NI	0	Yes	CR
8 (AA)	69/F	GC	25.5	I	0	0	0	0	Yes	CR
9 (AA)	46/F	GC	17.0	I	0	0	0	0	Yes	CR
10 (AA)	57/F	GC	17.0	I	0	0	0	0	Yes	CR
11 (AA)	25/F	GC	10.0	I	0	0	0	0	Yes	CR
12 (AA)	54/F	NF	30.0	I	0	0	0	0	Yes	CR
13 (AA)	36/F	GC	18.0	I	0	0	0	0	Yes	CR
14 (AA)	36/F	GC	19.0	I	0	0	NI	0	Yes	CR
15 (AA)	64/F	GC	23.0	I	0	0	NI	0	Yes	CR
16 (AA)	34/F	GC	12.8	I	0	0	0	0	Yes	CR
17 (AA)	63/F	GC	20.0	I	0	0	0	0	Yes	CR
18 (AA)	40/F	GC	25.0	I	0	0	0	0	Yes	CR
19 (AA)	30/F	GC	10.0	I	1	0	NI	0	Yes	CR
20 (AA)	48/F	GC	10.0	I	1	0	0	0	Yes	CR
21 (AA)	30/F	GC	15.0	I	2	0	0	0	Yes	CR
22 (AA)	51/F	NF	30.0	I	2	1	1	1	Yes	CR
23 (AA)	61/F	NF	45.3	II	0	0	0	0	Yes	CR
24 (AA)	40/F	A	62.0	II	1	0	1	1	Yes	CR
25 (AA)	39/F	GC	15.0	II	1	1	NI	0	Yes	CR
26 (AA)	51/F	NF	71.0	II	2	0	NI	0	Yes	CR
27 (AC)	28/F	GC + A	125.0	II	3	1	1	1	Yes	CR
28 (AC)	54/F	A	1,000.0	II	4	1	1	1	Yes	Metastases (32*)†
29 (AC)	44/F	GC + A	1,738.0	II	5	1	1	1	Yes	CR
30 (AC)	68/F	NF	230.0	II	6	1	1	1	Yes	Metastases (7*)†
31 (AC)	28/M	GC	400.0	IV	4	1	NI	1	No	Metastases†
32 (AC)	49/F	GC + A	1,550.0	IV	5	0	0	1	Yes	Metastases (95*)†
33 (AC)	79/F	GC	190.0	IV	5	1	0	1	No	Metastases†
34 (AC)	48/F	GC + A	860.0	IV	6	1	1	1	No	Metastases‡,†
35 (AC)	54/M	GC	1,255.0	IV	7	0	NI	1	Yes	Metastases (29*)†
36 (AC)	67/F	GC + A	230.0	IV	7	1	1	1	No	Metastases†
37 (AC)	26/M	GC + A	515.0	IV	7	1	1	1	No	Metastases†
38 (AC)	52/M	GC + A	827.0	IV	8	1	1	1	No	Metastases†
39 (AC)	26/F	GC + A	281.0	IV	8	1	1	0	No	Metastases†

Abbreviations: AA, adrenocortical adenoma; AC, adrenocortical carcinoma; F, female; M, male; GC, glucocorticoids; A, androgens; NF, nonfunctioning; NI, noninformative; CR, complete remission.
*Occurrence of metastases in months.
†Death.
‡Ovarian carcinoma metastasis.

functioning adrenocortical adenomas, suggesting an association with the tumor phenotype ($P = 0.01$, χ^2 test). Despite the higher frequency of β-catenin immunostaining alterations in adrenocortical carcinomas than in adrenocortical adenomas, the frequency of mutation [7 of 26 (27%) in adrenocortical adenomas; 4 of 13 (31%) in adrenocortical carcinomas] was similar in the two groups of tumors. The adrenocortical cancer cell line H295R has a similar mutation leading to alteration of Ser^{45} of β-catenin.

Activating *β-catenin* mutation in the human adrenocortical cancer H295R cell line leads to efficient T-cell factor–dependent transcription. Stimulation of Wnt signaling in the human adrenocortical cancer H295R cell line was monitored by transfection with a TCF reporter (pTOPFLASH) and a control plasmid containing scrambled TCF-binding sites (pFOPFLASH). Constitutive activity of the TCF reporter is observed in the H295R cell line transfected with the pTOPFLASH. This robust expression can be significantly down-regulated by adding the plasmid pΔNTCF4 encoding the dominant-negative form of TCF (Fig. 2). This result shows that activating *β-catenin* mutation in H295R cell line leads to efficient and constitutive TCF-dependent transcription.

Table 2. Mutation analysis and immunohistochemical staining of β-catenin in 39 patients with sporadic adrenocortical tumors and in human cell line H295R

Patients	Amino acid change	β-Catenin intracellular accumulation (focal/diffuse)
1 (AA)	–	–
2 (AA)	–	–
3 (AA)*	S45P	+ (focal)
4 (AA)*	S45P	+ (focal)
5 (AA)	–	–
6 (AA)	–	–
7 (AA)	–	–
8 (AA)	S45Y	+ (focal)
9 (AA)	S45P	+ (focal)
10 (AA)	–	–
11 (AA)	–	–
12 (AA)*	S45F	+ (focal)
13 (AA)	–	–
14 (AA)	–	–
15 (AA)	26,813 del 376 bp	+ (focal)
16 (AA)	–	+ (focal)
17 (AA)	–	–
18 (AA)	–	–
19 (AA)	–	–
20 (AA)	–	–
21 (AA)	–	–
22 (AA)*	–	+ (focal)
23 (AA)*	DinsS33S	+ (focal)
24 (AA)	–	–
25 (AA)	–	+ (focal)
26 (AA)*	–	–
27 (AC)	–	–
28 (AC)	–	+ (diffuse)
29 (AC)	–	+ (diffuse)
30 (AC)*	–	+ (diffuse)
31 (AC)	–	+ (diffuse)
32 (AC)	–	+ (diffuse)
33 (AC)	S45F	+ (diffuse)
34 (AC)	–	+ (diffuse)
35 (AC)	–	+ (diffuse)
36 (AC)	–	–
37 (AC)	del S45	+ (diffuse)
38 (AC)	T41A	+ (diffuse)
39 (AC)	26,993 del 489 bp	+ (diffuse)
H295R	S45P	+

*Nonfunctioning.

Discussion

Immunohistochemistry revealed abnormal cytoplasmic and/or nuclear accumulation of β-catenin, an evidence of activation of the Wnt signaling pathway, in 21 of the 39 adrenocortical tumors studied. An activating somatic mutation of the *β-catenin* gene was shown in 11 of these 21 adrenocortical tumors with abnormal β-catenin cellular localization. Similarly, the H295R adrenocortical cancer cell line also showed β-catenin protein accumulation associated with a point mutation of Ser^{45} in exon 3. Reporter assays showed that activating *β-catenin* mutation in the human adrenocortical cancer H295R cell line leads to efficient TCF-dependent transcription.

This is the first study, as far as we know, demonstrating that Wnt signaling activation is frequent in adrenocortical tumorigenesis. Blaker et al. (21), who studied one adrenocortical adenoma in a patient with familial adenomatous polyposis coli, did not describe any *β-catenin* mutation but found a somatic *APC* mutation. Semba et al. (22), in a study of adrenocortical tumors, mostly adenomas, did not find any nuclear accumulation of β-catenin; because this study only considered nuclear accumulation as a marker of an active β-catenin signaling pathway, exon 3 was not searched for genetic alterations.

Transcriptome analysis by microarray has shown that the potential target of the β-catenin pathway, *ENC-1*, is up-regulated in adrenocortical carcinomas, suggesting that Wnt signaling may play a role in adrenocortical tumorigenesis (23). Interestingly, in our series, most (8 of 12) of the activating *β-catenin* mutations

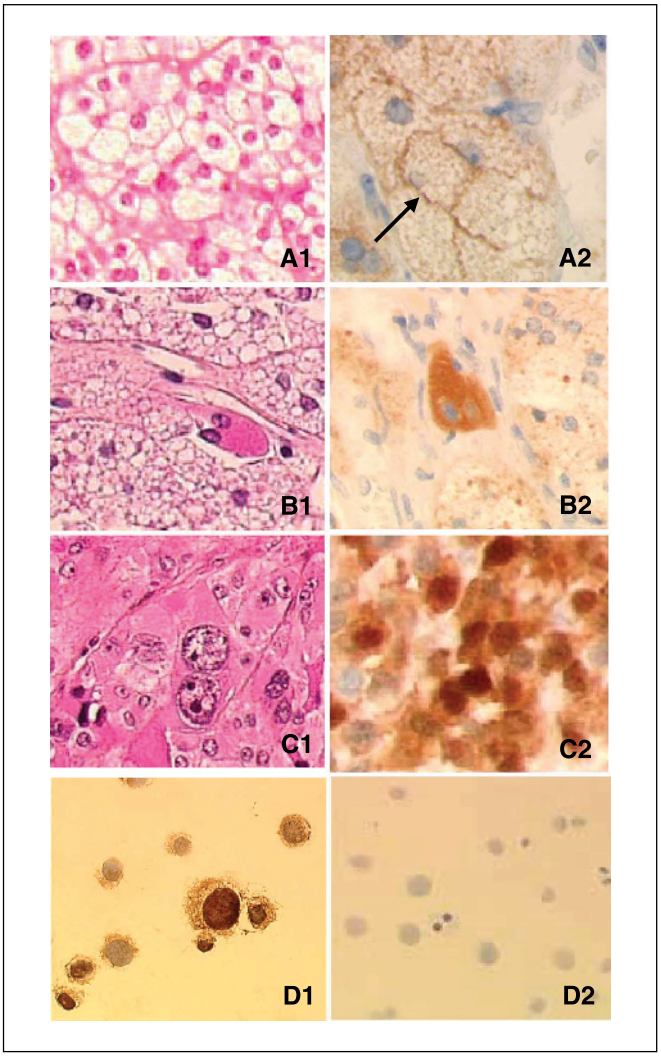

Figure 1. H&E-safran and immunohistochemical staining for β-catenin in adrenocortical tumors and the human adrenocortical carcinoma cell line H295R. Adrenocortical tumor with a Weiss score of 0 (adrenocortical adenoma) and without β-catenin mutation (*A1:* H&E-safran, 200×; *A2:* immunohistochemical staining of β-catenin, 400×; *arrow,* membranous localization of β-catenin). Adrenocortical tumor with a Weiss score of 0 (adrenocortical adenoma) and with a β-catenin mutation (*B1:* H&E-safran, 400×; *B2:* immunohistochemical staining of β-catenin, 400×). Adrenocortical tumor with a Weiss score of 8 (adrenocortical carcinoma) and with a β-catenin mutation (*C1:* H&E-safran, 400×; *C2:* immunohistochemical staining of β-catenin, 400×). Human cell line H295R (*D1:* immunohistochemical staining of β-catenin, 400×; *D2:* negative control, 400×).

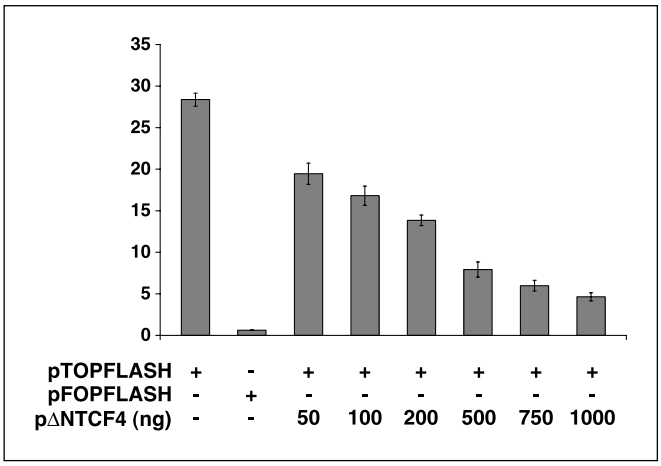

Figure 2. Study of β-catenin/TCF activity in the H295R cell line. Histogram showing the relative luciferase activity. Cells were transfected with 0.25 μg of pTOPFLASH (or pFOPFLASH) and 50, 100, 200, 500, 750, and 1,000 ng of pΔNTCF4 plasmid. *Columns,* mean; *bars,* SD. Experiments were repeated thrice with similar results.

involved the residue Ser^{45} (24–26) in the consensus GSK3-β/CK1 phosphorylation site. It would be interesting to elucidate the physiologic relevance of this mutation in adrenocortical tumorigenesis. In this study, transfections with a TCF reporter gene show constitutive overactivity of the β-catenin signaling pathway in the H295R cell line. Because this cell line harbors a Ser^{45} *β-catenin* mutation, this shows that in the adrenocortical cortex these genetic alterations lead to Wnt signaling pathway dysregulation and constitutive activation at the nuclear level.

The high rate [21 of 39 (54%)] of adrenocortical tumors with β-catenin alterations suggests that activation of the Wnt signaling pathway is the most prevalent defect in adrenocortical tumorigenesis. Mutations activating β-catenin are found in both adrenocortical adenomas and adrenocortical carcinomas. This is thus the first molecular defect to be reported with the same prevalence in both benign and malignant adrenocortical tumors. *β-Catenin* mutations have previously been reported in benign tumors, in particular in gallbladder adenomas, and were more frequent than in carcinomas (27). In contrast, *p53* mutations are found mainly in adrenocortical carcinomas and *PRKAR1A* mutations in adrenocortical adenomas. In adrenocortical adenomas, β-catenin alterations seem more prevalent in nonfunctioning than in functioning tumors. This suggests that β-catenin pathway activation might be involved in the development of nonsecreting adrenocortical adenomas and adrenocortical carcinomas. Similarly, loss of expression of the transcription factor cAMP-responsive element binding protein seems to be associated with less differentiated nonfunctioning or malignant adrenocortical tumors (28). In contrast, somatic mutations of *Gαs* or *PRKAR1A*, associated with activation of the cyclic AMP pathway, are observed in benign secreting adrenocortical tumors (6, 10, 29, 30).

Abnormal cellular localization of β-catenin is more frequent in adrenocortical carcinomas than in adrenocortical adenomas and was observed in more than 75% of adrenocortical carcinomas. This suggests that genetic alterations of other components of the Wnt signaling pathway, such as *APC* or *axin*, may also contribute to adrenocortical carcinomas (31, 32). Alternatively, the high frequency of β-catenin activation in adrenocortical carcinomas could be the result of cross talk with other signaling pathways, as for instance the IGF system which is almost always activated in such tumors (8). This hypothesis is particularly pertinent for adenocarcinomas of the gastroesophageal junction that frequently show nuclear β-catenin expression but very few APC and *β-catenin* mutations (33). As *β-catenin* mutation is the first molecular alteration identified in both adrenocortical adenomas and adrenocortical carcinomas, there may be a common pathophysiologic mechanism involving a multistep tumorigenesis process as speculated for other types of tumors (34). However, this multistep process would mainly concern nonfunctioning adrenal adenoma and adrenal carcinoma. The much more diffuse β-catenin immunostaining observed in adrenocortical carcinomas than in adrenocortical adenomas suggests that the progression towards a malignant phenotype involves other events leading to severe Wnt signaling pathway dysregulation in cancers.

Finally, the diffuse pattern of β-catenin immunostaining may serve as a diagnostic marker for malignancy in adrenocortical tumors, and indeed, this diagnosis is in some cases difficult with conventional pathologic methods.

In conclusion, this is the first study to show that Wnt signaling activation is frequent in adrenocortical tumors. It could be explained in half of the cases by somatic *β-catenin* mutations. These alterations are present in both malignant and benign adrenocortical tumors, and, in benign cases, seems to favor the development of nonfunctioning tumors. Functional studies in the H295R cell line showed that these genetic alterations of *β-catenin* lead to constitutive activity of the Wnt signaling pathway in adrenocortical tumors. Other genetic defects that could activate the Wnt signaling pathway are likely to be found in tumors that do not have a *β-catenin* mutation. This finding contributes to the development of new therapeutic approaches targeting the Wnt pathway in malignant adrenocortical tumors for which currently available medical therapies are very limited.

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