High Diagnostic and Prognostic Value of Steroidogenic Factor-1 Expression in Adrenal Tumors

Silviu Sbiera,* Sebastian Schmull,* Guillaume Assie, Hans-Ullrich Voelker, Luitgard Kraus, Melanie Beyer, Bruno Ragazzon, Felix Beuschlein, Holger S. Willenberg, Stefanie Hahner, Wolfgang Saeger, Jerome Bertherat, Bruno Allolio,* and Martin Fassnacht*

Department of Internal Medicine I (S.Sb., S.Sc., L.K., M.B., S.H., B.A., M.F.), Endocrine and Diabetes Unit, University Hospital Würzburg, and Department of Pathology (H.-U.V.), University of Würzburg, D-97080 Würzburg, Germany; Institut Cochin (G.A., B.R., J.B.), Endocrinology, Metabolism, and Cancer Department, Université Paris Descartes, F-75014 Paris, France; Department of Medicine/Endocrinology (F.B.), Medizinische Klinik Innenstadt, University of Munich, D-80336 Munich, Germany; Department of Endocrinology, Diabetes, and Rheumatology (H.S.W.), University Hospital Düsseldorf, D-40225 Düsseldorf, Germany; and Institute for Pathology (W.S.), Katholische Marienkrankenhaus, D-22087 Hamburg, Germany

Context: No immunohistochemical marker has been established to reliably differentiate adrenocortical tumors from other adrenal masses. A panel of markers like melan-A and inhibin-α is currently used for this purpose but suffers from limited diagnostic accuracy. We hypothesized that expression of steroidogenic factor-1 (SF-1), a transcription factor involved in adrenal development, is of value for the differential diagnosis of adrenal masses and predicts prognosis in adrenocortical carcinoma (ACC).

Patients and Methods: SF-1 protein expression was assessed by immunohistochemistry on tissue samples from 167 ACC, 52 adrenocortical adenomas (ACA), six normal adrenal glands, six normal ovaries and 73 neoplastic nonsteroidogenic tissues. In an independent cohort of 33 ACC and 58 ACA, SF-1 mRNA expression was analyzed. SF-1 expression was correlated with clinical outcome in patients with ACC.

Results: SF-1 protein staining was detectable in 158 of 161 (98%) evaluable ACC samples including 49 (30%) with strong SF-1 staining and in all normal and benign steroidogenic tissues. In addition, SF-1 mRNA expression was present in all 91 analyzed adrenocortical tumors. In contrast, SF-1 expression was absent in all nonsteroidogenic tumors. Strong SF-1 protein expression significantly correlated with poor clinical outcome: tumor stage-adjusted hazard ratio for death 2.46 [95% confidence interval (CI) = 1.30–4.64] and for recurrence 3.91 (95% CI = 1.71–8.94). Similar results were obtained in the independent cohort using RNA analysis [tumor stage-adjusted hazard ratio for death 4.69 (95% CI = 1.44–15.30)].

Conclusion: SF-1 is a highly valuable immunohistochemical marker to determine the adrenocortical origin of an adrenal mass with high sensitivity and specificity. In addition, SF-1 expression is of stage-independent prognostic value in patients with ACC. (***J Clin Endocrinol Metab*** **95: E161–E171, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-0653 Received March 18, 2010. Accepted June 8, 2010.
First Published Online July 21, 2010
* S.Sb. and S.Sc. are joint first authors; B.A. and M.F. are joint senior authors.

Abbreviations: ACA, Adrenocortical adenoma; ACC, adrenocortical carcinoma; CI, confidence interval; HR, hazard ratio; SF-1, steroidogenic factor-1; TMA, tissue microarray.

Adrenal masses are among the most frequent human tumors with a prevalence of at least 3% in a population over the age of 50 yr (1). Although the majority of these lesions represent benign adenomas, adrenocortical carcinomas and metastases amount to 5–10% of all tumors (1). Clinical symptoms and biochemical evidence of autonomous hormone excess may indicate the adrenocortical origin of the lesion. However, the differentiation of a nonfunctioning adrenocortical tumor from a metastasis of an extraadrenal malignancy or a nonsecretory pheochromocytoma is challenging. Due to their broad histomorphological heterogeneity, accurate typing of adrenal tumors often poses a major diagnostic problem, and conventional histology frequently offers no conclusive diagnosis of the origin of an individual neoplasm. Many malignant neoplasias metastasize to the adrenal gland including melanoma, breast, lung, renal, and gastrointestinal cancer, making this organ the fourth most common site of metastasis in humans (2, 3). Therefore, reliable immunohistochemical markers are required to establish the correct diagnosis. In 1990, Schröder *et al.* (4, 5) described a monoclonal antibody (D11) with high specificity for adrenocortical tissue. However, further studies suggested that immunoreactivity is observed only in the subset of well differentiated adrenocortical carcinoma (ACC) markedly limiting its use as a general marker for ACC (6–9). In addition, D11 is not commercially available and currently no longer accessible. Melan-A (MART-1) and inhibin-α were also suggested as putative markers for distinguishing primary from secondary adrenal tumors (10–13). However, they fail to recognize 28 and 31% of ACC, respectively (Table 1) (14–20). Thus, due to their limited sensitivity, none of the proposed markers for adrenocortical tumor has gained general acceptance (21, 22).

Already in 1995, Sasano *et al.* (7, 23) suggested steroidogenic factor-1 (SF-1; also known as Ad4BP and NR5A1) as a marker to differentiate between tumors of adrenocortical and nonadrenocortical origin. SF-1 is a transcription factor expressed primarily in the hypothalamus, pituitary, and steroidogenic organs like adrenal glands, testes, and ovaries. It plays a key role in the development of steroidogenic tissues (24, 25) and is involved in the regulation of steroid biosynthesis (26, 27).

Recent studies have demonstrated overexpression of SF-1 in most cases of childhood adrenocortical tumors (28, 29). Importantly, it was also shown that elevated levels of SF-1 lead to increased proliferation of human adrenocortical cells *in vitro* and to tumorigenesis in mice (30–32). Accordingly, SF-1-stimulated adrenocortical cell proliferation was inhibited *in vitro* by SF-1 inverse agonists (33). These observations suggest an important role of SF-1 in the pathogenesis of adrenocortical tumors. However, up to now, its value as a marker for ACC has been investigated only in small series of eight, five, and four ACC samples, respectively (34–36).

Accordingly, in this study, we investigated not only the role of SF-1 as a diagnostic tool in adrenal tumors but also its prognostic value in ACC.

Patients and Methods

Patients and tissue

German cohort

Three hundred four tissue samples (231 derived from steroidogenic and 73 from nonsteroidogenic tissue) were collected between 1989 and 2008. Samples of steroidogenic tissues comprised 167 ACC in different stages of disease, 52 benign adrenocortical adenomas (ACA), six normal adrenal glands, and six normal ovaries (Table 2). Nonsteroidogenic tissues included carcinomas derived from kidney (n = 11), lung (n = 12), breast (n = 8), colon (n = 7), pancreas (n = 5), liver (n = 7), prostate (n = 4), endometrium (n = 2), and ovary (n = 3) and eight pheochromocytomas, three melanoma metastases, two lymphomas, and one seminoma. All patients donating adrenocortical tissues gave written informed consent for collecting tissue and clinical data, and the study was approved by the ethics committee of the University of Würzburg. The other tissues derived from the tissue bank at the Department of Pathology at the University of Würzburg and were analyzed in an anonymous fashion in accordance with a general decision of the local ethics committee. For ACC patients, detailed clinical data, including follow-up and survival data, were collected in a structured manner by the German ACC Registry (37). Resection of the primary tumor was considered complete (R0 resection) if surgical, pathological, and imaging reports gave no evidence for remaining disease. Presence of distant metastases or recurrence was evaluated at the time of diagnosis and during follow-up visits by computerized tomography of chest and abdomen every 3–6 months. Patients with histologically confirmed ACA are assumed to be cured, and no structured follow-up is recommended (38). Accordingly, in 38 of 52 patients, in whom clinical outcome data were available, no evidence for recurrence was found.

French cohort

An independent cohort of adrenocortical tumors (n = 91) derived from a French series (39). Only samples with undoubtful histological diagnosis and evaluable RNA microarray data were included (ACA n = 58, ACC n = 33). These tumors were prospectively collected by the COMETE network between 1993 and 2005. Patient characteristics and collection of clinical data are described in detail elsewhere (39–41). Written informed consent was obtained from all patients, and the study was approved by the institutional review board of the Cochin Hospital (Paris).

RNA extraction and analysis

RNA extraction and analysis was performed as described recently (39). Microarray analyses were performed using 3 μg total RNA in each sample as starting material following the manufacturer's protocol, as previously described (39). The labeled cDNA was hybridized to HG-U133 Plus 2.0 Affymetrix GeneChip arrays (Affymetrix, Santa Clara, CA), and the chips were scanned with GCOS version 1.4.

TABLE 1. Overview of immunohistochemical markers for the diagnosis of adrenocortical tumors

Reference	ACC		ACA		NAG		Nonadrenocortical tumors	
	+	−	+	−	+	−	+	−
D11 (nuclear staining)								
Komminoth *et al.*, 1995 (8)	22	5			13	0	0	28
Schröder *et al.*, 1990 (4)	9	0	40	0	27	0	1	143
Schröder *et al.*, 1992 (5)	15	0	57	0				
Tartour *et al.*, 1993 (6)	8	10			3	0	0	103
Wajchenberg *et al.*, 2000 (9)	22	13	38	0	27	17		
Total	76	28	135	0	70	17	1	274
For ACC: sensitivity, 73%; specificity, 99%; positive predictive value, 98%; negative predictive value, 90%								
Inhibin-α (cytoplasmic staining)								
Arola *et al.*, 2000 (16)	23	7	46	19	10	0	0	20
Fetsch *et al.*, 1999 (17)	7	0	15	0			0	23
Jalali and Krishnamurthy, 2005 (15)	1	4	4	31			0	10
McCluggage *et al.*, 1998 (12)	4	0	15	0	20	0	0	15
Munro *et al.*, 1999 (13)	10	2	17	0	6	0		
Pan *et al.*, 2005 (14)	27	13	52	10			6	787
Pelkey *et al.*, 1998 (18)	12	3	18	5			2	52
Zhang *et al.*, 2003 (19)	0	5	51	10	3	0	0	5
Zhang *et al.*, 2004 (20)	28	17	50	16	23	0	0	47
Total	112	51	268	91	62	0	8	949
For ACC: sensitivity, 69%; specificity, 99%; positive predictive value, 93%; negative predictive value, 95%								
A103 (Melan-A) (cytoplasmic staining)								
Busam *et al.*, 1998 (10)	29	0	5	0			7	185
Ghorab *et al.*, 2003 (11)	10	1	21	0			1	142
Jalali and Krishnamurthy 2005 (15)	4	1	32	3			0[a]	8
Pan *et al.*, 2005 (14)	22	18	60	2			3	790
Zhang *et al.*, 2003 (19)	0	5	55	6	3	0	0	5
Zhang *et al.*, 2004 (20)	35	13	60	6	23	0	0[a]	41
Total	100	38	233	17	26	0	11	1171
For ACC: sensitivity, 72%; specificity, 99%; positive predictive value, 90%; negative predictive value, 97%								

NAG, Normal adrenal gland.

[a] Melanoma samples were excluded from the analysis.

TABLE 2. Adrenocortical tissues: patients and tumor characteristics

	Age [yr (SD)]	Sex (male/female)	Size of the adrenal tumor [cm (SD)]
German series			
Normal adrenal gland (n = 6)	59 (17)	4/2	
ACA			
Aldosterone-producing adenoma (n = 26)	53 (11)	17/9	1.7 (0.9)
Cortisol-producing adenoma (n = 16)	50 (12)	4/12	3.4 (1.5)
Hormonally inactive adenoma (n = 10)	64 (11)	6/4	3.9 (3.0)
ACC[a]			
Primary tumor (n = 133[b])	49 (16)	48/85	12 (4.4)
ENSAT stage I (n = 5)	54 (24)	2/3	4.7 (0.3)
ENSAT stage II (n = 48)	48 (17)	19/29	11.9 (4.5)
ENSAT stage III (n = 40)	53 (14)	14/27	11.8 (3.6)
ENSAT stage IV (n = 34)	47 (18)	11/23	13.4 (4.4)
Local recurrence (n = 19)	46 (17)	9/10	
Distant metastases (n = 15)	44 (11)	3/12	
French series			
ACA			
Aldosterone-producing adenoma (n = 10)	48 (12)	4/6	2.5 (0.7)
Cortisol-producing adenoma (n = 29)	43 (11)	3/26	3.8 (1.6)
Hormonally inactive adenoma (n = 19)	54 (11)	3/16	4.2 (1.5)
ACC[a]			
ENSAT stage I (n = 1)	41	0/1	5
ENSAT stage II (n = 17)	44 (19)	1/16	11.1 (4.9)
ENSAT stage III (n = 5)	48 (5)	2/3	15.0 (1.4)
ENSAT stage IV (n = 10)	47 (19)	4/6	11.6 (3.2)

Data represent mean values (SD) or number.

[a] Tumor stage at the time of diagnosis was reported according to the European Network for the Study of Adrenal Tumors (ENSAT) classification (49).

[b] In six cases, tumor stage was not determined. Forty-eight ACC were cortisol secreting, 13 exclusively sex hormone or precursors secreting, seven aldosterone secreting, and 18 hormonally inactive. In 48 cases, no sufficient endocrine work-up was performed preoperatively or data were not available.

Immunohistochemistry

Tissue samples from 167 ACC, 15 ACA, five normal adrenal glands, and 10 nonadrenocortical malignancies were assembled into three tissue microarrays (TMA) as recently described (42–44). Twenty-eight samples included in the TMA were analyzed as standard full sections to validate the array results. In addition, 107 tissue samples including one normal adrenal gland, six normal ovaries, 37 adrenal adenomas, and 63 nonsteroidogenic tumors were processed as standard full sections.

TMA and full sections were deparaffinized twice in 100% xylene (Sigma-Aldrich, Seelze, Germany) for 10 min with rehydration twice in 100 and 70% ethanol, followed by an extensive washing step with distilled water. Antigen retrieval was performed in 10 mM citric acid monohydrate buffer (pH 6) twice for 5 min at 750 W in a microwave oven. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide/methanol solution for 10 min. Subsequently, blocking of unspecific protein-antibody interactions was performed with 20% human AB serum in Dulbecco's Phosphate-Buffered Saline (DPBS) for 1 h at room temperature. SF-1 protein was detected by incubation with monoclonal mouse antihuman SF-1 antibody (Perseus-Proteomics, Tokyo, Japan) 1:100 in DPBS for 17 h, with the reaction controlled with N-Universal Negative Control Mouse (Dako, Glostrup, Denmark). Signal amplification was achieved by EnVision+ System Labeled Polymer-HRP Anti-Mouse (Dako) for 30 min and developed for 7 min with NovaRED Substrate Kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Nuclei were counterstained with Mayer's hemalaun for 2 min.

Microscopic analysis

All slides were analyzed independently by two investigators. TMA samples were included in the analysis only if two or more evaluable cores were available after the staining procedure. Only nuclear staining was evaluated (23), and staining intensity was graded as negative (0), low to medium (1), or strong (2). The percentage of positive tumor cells was calculated for each specimen and scored 0 if 0% were positive, 0.1 if 1–9%, 0.5 if 10–49% and 1 if 50% or more. A semiquantitative H-score was then calculated by multiplying the staining intensity grading score with the proportion score as described (45). Where discrepancies were observed, results were double checked by both investigators together with a third observer. Samples of the TMA without staining were also reanalyzed in full tissue sections. Normal adrenal gland sections served as positive controls during all staining procedures and cells of the tumor stroma as internal negative control.

Statistics

Characteristics of tumors and patients are presented as means with their respective SD values for normally distributed variables. The interobserver agreement for the scoring system was evaluated using Cohen's κ-coefficient and confirmed using Pearson's correlation coefficient. As cutoff for strong agreement of 0.81 was chosen for the κ-coefficient and 0.75 for Pearson's coefficient (46, 47). Categorical variables were compared by Fisher's exact test and χ^2 test. Correlation between endocrine activity of the tumor and SF-1 expression was analyzed using Spearman's correlation test. Affymetrix microarray data were normalized

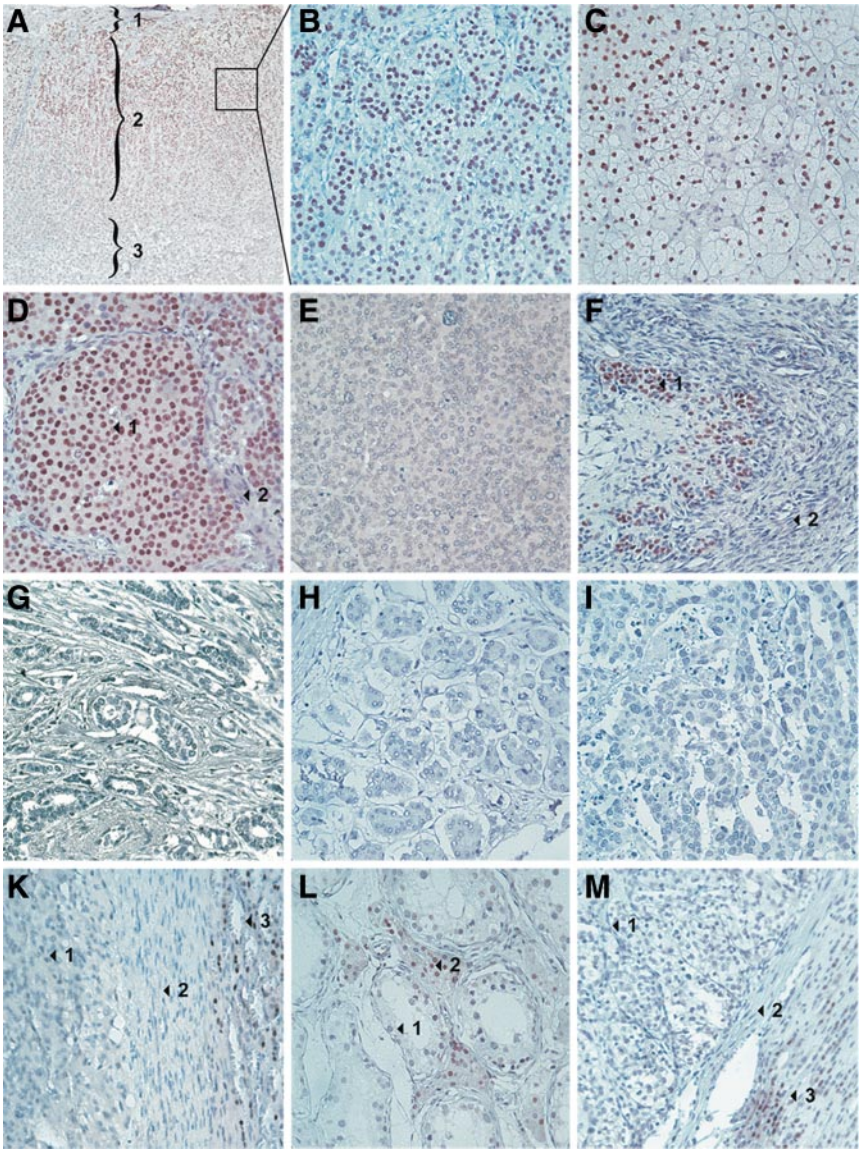

FIG. 1. SF-1 protein expression in normal, benign, and malignant steroidogenic (A–F) and nonsteroidogenic neoplastic tissues (G–M): A, normal adrenal gland, including capsule (A-1), cortical zones (A-2, and magnification in B), and medulla (A-3); C, adrenocortical adenoma; D and E, adrenocortical carcinoma (D-1, tumor cells; D-2, fibroblasts); F, normal ovary; G, breast carcinoma; H, pancreas carcinoma; I, endometrium carcinoma; K, pheochromocytoma; L, intratubular germ cell neoplasia, including SF-1-positive Leydig cells (L-2); M, renal clear cell carcinoma, including adjacent adrenocortical cells (M-3).

using the robust multiarray averaging method (48). Survival analysis for ACC patients was calculated using the Kaplan-Meier method, and differences between groups were assessed with log-rank and Cox proportional hazards statistics. Overall survival was defined as time elapsed from primary resection of ACC to death or last follow-up visit. Recurrence-free survival was analyzed only in patients with European Network for the Study of Adrenal Tumors stages I–III (49) after complete resection and was defined as time from the date of tumor resection to the first evidence of relapse or last follow-up without evidence for disease. The Cox proportional hazards model was used for multivariate analysis to test the influence of sex, age, tumor stage, and endocrine activity of the tumors on SF-1-related survival. In the French ACC cohort, Cox analysis was used to determine tumor stage-adjusted survival with SF-1 RNA expression as a continuous variable. Subsequently, a Kaplan-Meier regression analysis was performed using dichotomized categorical SF-1 values (cutoff value for this data set = 5.78).

Results are given as hazard ratios (HR) including 95% confidence interval (CI). The significance level was set at $\alpha = 5\%$ for all comparisons. All statistical tests were performed using the SPSS software package (version 15.0.0; Chicago, IL).

Results

Immunohistochemical detection of SF-1 protein

As shown in Fig. 1, SF-1 staining was localized to the nucleus and was homogeneously distributed both in standard tissue slides and among the different cores from the same tissue sample in the TMA. Interobserver agreement for assessing SF-1 expression was strong with a κ-coefficient of 0.92 (95% CI = 0.87–0.97) and Pearson's correlation coefficient 0.86 (95% CI = 0.82–0.89). In agreement with previous publications (50), SF-1 was expressed in normal steroidogenic tissue (adrenal gland n = 6 of 6 and ovary n = 6 of 6). Within the adrenal gland, its expression was restricted to the cortex (Fig. 1A-2) with strong staining in the zona glomerulosa and zona fasciculata (Fig. 1B) and low staining intensity in the zona reticularis. No staining was observed in the capsular tissue and the medulla (Fig. 1A-1 and -2). In normal ovaries, strong SF-1 expression was detectable in theca and granulosa cells (Fig. 1F-1) but not in other cell types (Fig. 1F-2).

SF-1 was expressed in all benign adrenocortical neoplasias (Fig. 1C and Table 3). In ACC, SF-1 expression was detectable in 158 of 161 evaluable tissue samples (98%), and in 30% of samples (n = 49), staining intensity was strong (Fig. 1, D and E). In both ACA and ACC, SF-1 staining pattern was independent of endocrine activity or the origin (primary tumor, local recurrence, or distant metastases) of the sample (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Accordingly, in the French cohort, SF-1mRNA expression was found in all 91 adrenocortical tumors using microarray analysis.

TABLE 3. SF-1 expression scores distribution in steroidogenic and nonsteroidogenic tissues

Tissue	n	SF-1 expression (H-score) (n)		
		Negative (0)	Low (1)	High (2)
Normal and benign steroidogenic tissues	64	0 (0%)	26 (41%)	38 (59%)
Normal adrenal gland[a]	6	0	3	3
Normal ovary	6	0	6	0
Inactive ACA	10	0	2	8
Aldosterone-producing adenoma	26	0	9	17
Cortisol-producing adenoma	16	0	6	10
ACC	161	3 (2%)	109 (68%)	49 (30%)
Primary tumor	130	3	84	43
Local recurrence	18	0	14	4
Distant metastases	13	0	11	2
Nonsteroidogenic tumors	73	73 (100%)	0 (0%)	0 (0%)
Breast carcinoma	8	8	0	0
Colon carcinoma	7	7	0	0
Hepatocellular carcinoma	7	7	0	0
Endometrial carcinoma	2	2	0	0
Melanoma metastasis	3	3	0	0
Non-Hodgkin lymphoma	2	2	0	0
Non-small-cell lung carcinoma	11	11	0	0
Ovarian carcinoma	4	4	0	0
Pancreatic carcinoma	5	5	0	0
Pheochromocytoma	8	8	0	0
Prostate carcinoma	4	4	0	0
Renal cell carcinoma	11	11	0	0
Seminoma	1[b]	1	0	0
Small-cell lung carcinoma	1	1	0	0

[a] Only adrenocortical cells were positive, whereas cells of the medulla were negative.

[b] Only Leydig cells were positive.

In contrast, in none of the 73 tissues derived from nonsteroidogenic organs was SF-1 staining detectable (Table 3 and Fig. 1, G–M). The sample selection included all tumor entities that typically metastasize to the adrenal gland and pheochromocytomas that are sometimes difficult to distinguish histologically from ACC. Of note, Leydig cells within a seminoma (Fig. 1L-2) were SF-1 positive, whereas tumor cells were negative (Fig. 1L-1). Similarly, in a renal cell carcinoma, tumor cells were SF-1 negative (Fig. 1M-1), whereas invaded adrenocortical tissue showed strong SF-1 expression (Fig. 1M-3).

By analyzing all ACC samples and all tumor tissues derived from nonsteroidogenic organs, the sensitivity, specificity, positive predictive value, and negative predictive value for SF-1 in detecting ACC were 98.6, 100, 100, and 97.3%, respectively.

Correlation of SF-1 expression and clinical outcome in patients with ACC

For survival analysis, only patients with tumor samples from primary surgery and sufficient follow-up data were included (n = 130). SF-1 expression was absent in only three, and SF-1 staining intensity was low in 84 and strong in 43 samples. In the high SF-1 expression group, 37 of 43 (86%) patients died from ACC and 39 of 43 (90%) had a tumor recurrence compared with 43 (51%) and 50 (59%) of 84 in the low SF-1 expression group. Median overall survival was 14 months (95% CI = 9.9–18.1 months) in patients with strong SF-1 staining, whereas it was 49.8 months (95% CI = 5.96–93.7 months) in patients with low SF-1 staining intensity (Fig. 2A). Univariate analysis revealed a significant association between strong SF-1 expression and mortality [HR for death = 2.46 (95% CI = 1.57–3.87], and in a multivariate model adjusted for tumor stage, sex, age, and endocrine activity of the tumors, the prognostic value of strong SF-1 expression remained unchanged [HR for death 2.46 (95% CI = 1.30–4.64); Table 4], confirming SF-1 expression as prognostic factor for survival in ACC.

Similar findings were found in the French ACC cohort using RNA microarray analysis. In this independent series, tumor stage-adjusted HR for death for continuous SF-1 values was 2.78 (95% CI = 1.32–5.86) and for dichotomized SF-1 values 4.69 (95% CI = 1.44–15.30; Fig. 2B).

In addition, we assessed the prognostic value of SF-1 expression after complete resection (n = 48). Median recurrence-free survival was significantly shorter in patients with strong SF-1 staining [8.8 months (95% CI = 7.7–9.9) *vs.* 37.7 months (95% CI = 9.8–65.6); Fig. 2C]. Again, multivariate analysis confirmed SF-1 expression as a prognostic factor [HR for recurrence = 3.91 (95% CI = 1.71–8.94); Supplemental Table 2].

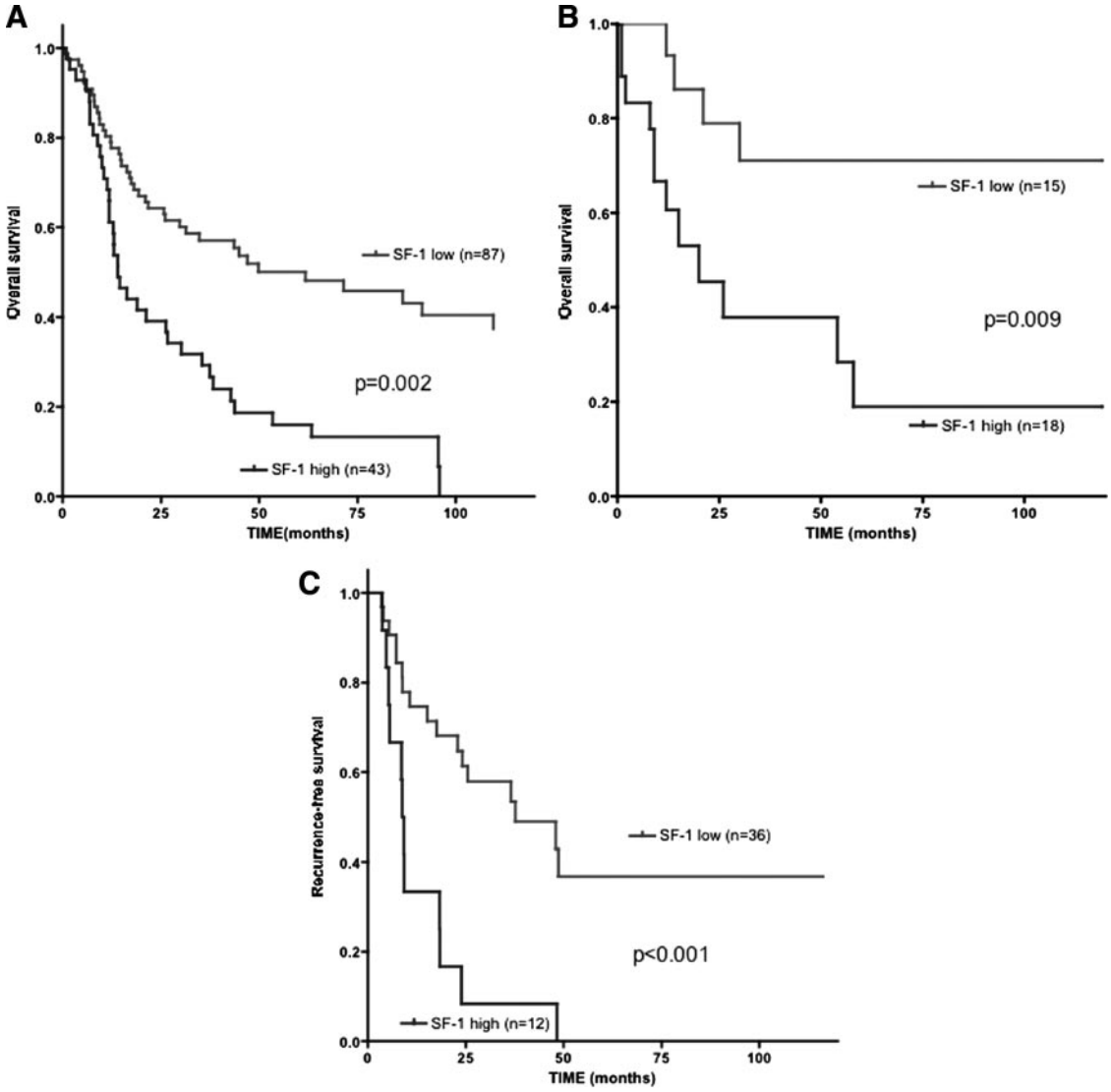

FIG. 2. Kaplan-Meier estimates on overall survival (A and B) and recurrence-free survival (C) in patients with adrenocortical carcinoma according to SF-1 expression. A, Overall survival of all ACC patients of the German cohort with tumor samples from primary surgery (n = 130); B, overall survival of ACC patients of the French cohort (n = 33); C, recurrence-free survival only in the subgroup of German patients in whom complete resection was achieved (n = 48). SF-1 expression was determined by immunohistochemistry (A and C) and RNA microarrays (B) (for details see *Patients and Methods*).

Discussion

Our large study including more than 161 evaluable ACC tissue samples indicates that SF-1 is a highly valuable immunohistological marker for the differential diagnosis of adrenal tumors. Only 2% of the 161 ACC samples, but all 73 nonadrenocortical tumors, were SF-1 negative, demonstrating that SF-1 has a much higher sensitivity and specificity to determine the adrenocortical origin of an adrenal lesion than has been reported for other immunohistological markers (Table 1). In addition, we provide the first evidence that high SF-1 expression is associated with poor clinical outcome in adults with ACC.

Histological diagnosis of ACC is often difficult, because ACC is a rare, morphologically heterogeneous tumor, and histomorphological criteria for the discrimination from pheochromocytoma or metastases from extraadrenal neoplasias are not well established (7, 22, 51, 52). For all immunohistological markers proposed in the past such as D11, melan-A, or inhibin-α, the percentage of negative ACC samples were at least 25%, and the total number of ACC samples for each antibody was 163 at maximum (Table 1).

Expression of nuclear receptor SF-1 in adrenocortical cells has been documented previously (50), and its important role in adrenal development is well established (27). Accordingly, adrenocortical cells in normal adrenal glands as well as all benign adrenal adenomas were positive for SF-1 staining. Already in 1995, SF-1 had been suggested to be a useful marker in the differential diagnosis of ACC (23), but the results of only 17 ACC samples stained with SF-1 antibody were published (34–36). In addition, for many years, no re-

TABLE 4. Factors influencing overall survival according to univariate and multivariate analyses in patients with ACC (German cohort)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age[a]	1.00	0.99–1.02	0.509	1.01	0.99–1.02	0.340
Sex						
Male (n = 47)[b]						
Female (n = 83)	0.90	0.57–1.40	0.627	0.57	0.31–1.01	0.055
Tumor stage						
I-II (n = 53)[c]						
III (n = 40)	1.62	0.92–2.83	0.089	1.81	0.90–3.65	0.094
IV (n = 34)	3.94	2.25–6.88	<0.001	4.37	2.15–8.87	<0.001
Hormone secretion						
Nonsecreting (n = 17)[d]						
Glucocorticoids (n = 48)	1.12	0.56–2.20	0.753	1.02	0.48–2.15	0.966
Sex hormones (n = 13)	0.98	0.39–2.45	0.967	1.38	0.53–3.60	0.516
Mineralocorticoids (n = 6)	2.03	0.70–5.91	0.193	0.906	0.27–3.06	0.874
SF-1						
Negative + low (n = 87)						
High (n = 43)	2.46	1.57–3.87	<0.001	2.46	1.30–4.64	0.006

For survival analyses, only patients with tumor samples from primary surgery and sufficient clinical data (including follow-up) were included (n = 130).

[a] Age HR associated with one unit increase in the predictor.

[b] Male sex was taken as the reference category.

[c] Stage I-II was the reference category.

[d] Nonsecreting ACC was the reference category (in 46 patients, no information was available).

liable antibody against human SF-1 for paraffin-embedded tissue was available. In contrast, the antibody used in our study is now easily available for purchase.

The fact that two of the three ACC samples without SF-1 immunoreactivity were hormonally active (Supplemental Table 1) may point to methodological limitations of immunohistochemistry in cases of low SF-1 expression. This view is supported by the consistent SF-1 mRNA expression in all adrenocortical neoplasias in the French cohort. However, it is also possible that a small percentage of ACC are truly SF-1 negative, potentially harboring activating mutations downstream in the SF-1 pathway. This hypothesis has to be addressed in further investigations analyzing downstream targets of SF-1 (31).

In our study, detection of SF-1 staining in nonadrenal tissue was restricted to organs that are well known for SF-1 expression (53–55). In contrast, none of the investigated pheochromocytomas or tumors that usually metastasize to the adrenal gland revealed positive immunoreactivity against SF-1. In addition, SF-1 staining of Leydig cells within a seminoma and of adrenocortical cells adjacent to renal cancer (Fig. 1) demonstrates the accuracy of the method. Taken together, our results strongly confirm the hypothesis by Sasano *et al.* (7) that SF-1 is the best available marker for adrenocortical tumors. In our view, it, therefore, should become part of the routine diagnostic workup of adrenal tumors.

The second important finding of our study is the correlation of SF-1 expression and clinical outcome in patients with ACC. It has been previously shown that the *SF-1* gene is amplified and overexpressed in childhood ACC (28, 29, 31) and that increased SF-1 dosage increases proliferation, decreases apoptosis of human adrenocortical cells, and induces adrenocortical tumors in transgenic mice (28, 29, 31). In addition, in adult ACC, chromosomal gains in 9q, where the SF-1 gene is located, have been described (56), suggesting that gene amplification may be the basis of SF-1 overexpression also in adult ACC. Therefore, there is increasing evidence that SF-1 dosage is critical for adrenal tumorigenesis (57). This concept is greatly supported by the demonstration of a reduced recurrence-free and overall survival in adult ACC patients exhibiting high SF-1 expression. The fact that this effect remains significant after adjusting for tumor stage and is also present in the French series indicates that SF-1 is not only a helpful diagnostic tool but also of important prognostic value.

At first glance, it seems to be a paradox that SF-1 is equally expressed in benign and malignant adrenocortical neoplasms and yet has such a strong and consistent association with prognosis in ACC. However, the action of SF-1 clearly varies depending on the cellular context. In differentiated adrenocortical cells, the major role of SF-1 is related to steroidogenesis with most of the steroidogenic enzymes possessing SF-1 response elements in their promoter (26, 27). However, SF-1 also plays a major role in fetal adrenal development by stimulating adrenal growth independent of its action on steroidogenesis (24, 25, 27). We hypothesize that in ACC, the cellular context resembles more the fetal phe-

notype, whereas benign tumors represent a differentiated phenotype. In agreement with this hypothesis is the fact that the level of SF-1 expression did not correlate with hormonal activity in our cohort. This view is further supported by the well-established overexpression of IGF-II in ACC (58). High IGF-II expression plays an important role in normal fetal adrenal development but is not found in adult adrenals or benign adrenocortical neoplasms (59–61). Thus, although SF-1 is obviously not causative for malignancy, it nonetheless may be a prerequisite for proliferation in ACC by providing a specific growth advantage on the background of oncogenic mutations. Similar molecular mechanisms have been described in other malignancies (62).

Currently, tumor stage is the only widely accepted prognostic marker in ACC. Few other markers have been suggested in the past for this purpose (44, 63, 64), but either their clinical value could not be confirmed or the immunohistochemical detection method is not established on a routine basis. Therefore, more reliable prognosis markers are needed to advise patients with ACC and a given tumor stage. Accordingly, if SF-1 immunohistochemistry will become part of the work-up in adrenal tumors, also prognostic information will be generated that may help to guide therapeutic decisions. This might be of special importance in patients after complete surgical resection, in whom a benefit of adjuvant therapy with mitotane (65) or radiotherapy of the tumor bed (66) has been suggested based on retrospective studies but is not yet generally accepted (67).

The negative association between SF-1 staining intensity and survival strengthens the concept of SF-1 being a crucial, dosage-dependent survival factor in this tumor entity. Therefore, SF-1 seems to be also an intriguing target for a therapeutic approach. Recently, SF-1 inverse agonists have been developed (68–70) and led to growth retardation of ACC cells (33), suggesting that these agonists might become useful future tools for treating ACC.

In conclusion, our study indicates that SF-1 is the best available immunohistological marker to determine the adrenocortical origin of an adrenal mass with high sensitivity and specificity. In addition, SF-1 expression is of stage-independent prognostic value in patients with ACC, suggesting that SF-1 plays an important role in the pathogenesis of this disease.

Acknowledgments

The advice of Dr. Hironobu Sasano (Department of Pathology, Tohoku University, School of Medicine and Tohoku University Hospital, Sendai, Japan) in selecting the appropriate SF-1 antibody and dilution is greatly acknowledged.

This study was part of the German adrenal network GANIMED (German Adrenal Network Improving Treatment and Medical Education). We are grateful to all colleagues who provided tumor material and clinical data for the German ACC registry. The following pathologists provided tumor material from two or more patients for the tissue array: Gerhard Seitz (Klinikum Bamberg), Harald Stein, Manfred Dietel, (Charite University Berlin), Gerhard Mall (Klinikum Darmstadt), Helmut Erich Gabbert (University Hospital Düsseldorf), Werner Schmid (University of Essen), Steffen Hauptmann (Martin-Luther University of Halle), Peter Schirrmacher (University of Heidelberg), Alfred C. Feller (University of Luebeck), C. James Kirkpatrick (University of Mainz), Roland Moll (University of Marburg), Cyrus Tschahargane (Lukaskrankenhaus, Neuss), Rainer Horst Krech (Klinikum Osnabrueck), Ferdinand Hofstaedter (University of Regensburg), and Andrea M. Gassel (Leopoldina Hospital Schweinfurt). The following hospitals/clinicians contributed clinical data from three or more investigated patients: Marcus Quinkler, Wolfgang Oelkers (University Hospital Charite Berlin), Peter Langer (University Hospital of Marburg), Christian Fottner (University Hospital Mainz), Michael Brauckhoff (University Hospital Halle), Horst L. Fehm (University Hospital Luebeck), Dagmar Führer (University Hospital Leipzig), and Stephan Petersenn (University Hospital Essen). We appreciate the support of Uwe Maeder (Tumor Center, University Hospital Wuerzburg) in establishing the German ACC registry database and are thankful to Michaela Haaf for documentation.

Address all correspondence and requests for reprints to: Dr. Martin Fassnacht, M.D., Endocrine and Diabetic Unit, Department of Internal Medicine I, University Hospital of Wuerzburg, Oberdürrbacher Strasse 6, D-97080 Würzburg, Germany. E-mail: fassnacht_m@medizin.uni-wuerzburg.de.

This work was supported by grants of the Deutsche Krebshilfe (Grant 107111 to M.F. and Grant 106 080 to B.A. and M.F.), the Deutsche Forschungsgemeinschaft (Grant FA 466/3-1 to M.F.), and the German Ministry of Research BMBF (Grant 01KG0501 to B.A. and M.F.).

Parts of the manuscript have been presented as oral presentation at the 52nd Annual Meeting of the German Endocrine Society, Giessen, Germany 2009, and as a poster at The Endocrine Society's 91st Annual Meeting, Washington, DC, 2009.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Grumbach MM, Biller BM, Braunstein GD, Campbell KK, Carney JA, Godley PA, Harris EL, Lee JK, Oertel YC, Posner MC, Schlechte JA, Wieand HS** 2003 Management of the clinically inapparent adrenal mass ("incidentaloma"). Ann Intern Med 138:424–429
2. **Abrams HL, Spiro R, Goldstein N** 1950 Metastases in carcinoma; analysis of 1000 autopsied cases. Cancer 3:74–85
3. **Lloyd RV, Kawashima A, Tischler AS** 2004 Tumours of the adrenal gland: secondary tumours. In: DeLillis RA, Lloyd RV, Heitz PU, Eng C, eds. Pathology and genetics: tumours of endocrine organs. 3rd ed. Lyon, France: IARC Press; 172–173
4. **Schröder S, Niendorf A, Achilles E, Dietel M, Padberg BC, Beisiegel U, Dralle H, Bressel M, Klöppel G** 1990 Immunocytochemical differential diagnosis of adrenocortical neoplasms using the monoclonal antibody D11. Virchows Arch A Pathol Anat Histopathol 417: 89–96
5. **Schröder S, Padberg BC, Achilles E, Holl K, Dralle H, Klöppel G** 1992 Immunocytochemistry in adrenocortical tumours: a clinico-

morphological study of 72 neoplasms. Virchows Arch A Pathol Anat Histopathol 420:65–70
6. **Tartour E, Caillou B, Tenenbaum F, Schröder S, Luciani S, Talbot M, Schlumberger M** 1993 Immunohistochemical study of adrenocortical carcinoma. Predictive value of the D11 monoclonal antibody. Cancer 72:3296–3303
7. **Sasano H, Suzuki T, Moriya T** 2006 Recent advances in histopathology and immunohistochemistry of adrenocortical carcinoma. Endocr Pathol 17:345–354
8. **Komminoth P, Roth J, Schröder S, Saremaslani P, Heitz PU** 1995 Overlapping expression of immunohistochemical markers and synaptophysin mRNA in pheochromocytomas and adrenocortical carcinomas. Implications for the differential diagnosis of adrenal gland tumors. Lab Invest 72:424–431
9. **Wajchenberg BL, Albergaria Pereira MA, Medonca BB, Latronico AC, Campos Carneiro P, Alves VA, Zerbini MC, Liberman B, Carlos Gomes G, Kirschner MA** 2000 Adrenocortical carcinoma: clinical and laboratory observations. Cancer 88:711–736
10. **Busam KJ, Iversen K, Coplan KA, Old LJ, Stockert E, Chen YT, McGregor D, Jungbluth A** 1998 Immunoreactivity for A103, an antibody to melan-A (Mart-1), in adrenocortical and other steroid tumors. Am J Surg Pathol 22:57–63
11. **Ghorab Z, Jorda M, Ganjei P, Nadji M** 2003 Melan A (A103) is expressed in adrenocortical neoplasms but not in renal cell and hepatocellular carcinomas. Appl Immunohistochem Mol Morphol 11: 330–333
12. **McCluggage WG, Burton J, Maxwell P, Sloan JM** 1998 Immunohistochemical staining of normal, hyperplastic, and neoplastic adrenal cortex with a monoclonal antibody against α-inhibin. J Clin Pathol 51:114–116
13. **Munro LM, Kennedy A, McNicol AM** 1999 The expression of inhibin/activin subunits in the human adrenal cortex and its tumours. J Endocrinol 161:341–347
14. **Pan CC, Chen PC, Tsay SH, Ho DM** 2005 Differential immunoprofiles of hepatocellular carcinoma, renal cell carcinoma, and adrenocortical carcinoma: a systemic immunohistochemical survey using tissue array technique. Appl Immunohistochem Mol Morphol 13:347–352
15. **Jalali M, Krishnamurthy S** 2005 Comparison of immunomarkers for the identification of adrenocortical cells in cytology specimens. Diagn Cytopathol 33:78–82
16. **Arola J, Liu J, Heikkilä P, Ilvesmäki V, Salmenkivi K, Voutilainen R, Kahri AI** 2000 Expression of inhibin alpha in adrenocortical tumours reflects the hormonal status of the neoplasm. J Endocrinol 165:223–229
17. **Fetsch PA, Powers CN, Zakowski MF, Abati A** 1999 Anti-α-inhibin: marker of choice for the consistent distinction between adrenocortical carcinoma and renal cell carcinoma in fine-needle aspiration. Cancer 87:168–172
18. **Pelkey TJ, Frierson Jr HF, Mills SE, Stoler MH** 1998 The α subunit of inhibin in adrenal cortical neoplasia. Mod Pathol 11:516–524
19. **Zhang H, Liu W, Wang X, Li G, Guo J, Li F, Liao D** 2003 [The expression of A103 and inhibin α in adrenocortical adenoma by high-throughput tissue microarray techniques]. Sichuan Da Xue Xue Bao Yi Xue Ban 34:424–426 (Chinese)
20. **Zhang HY, Wang XJ, Liu WP, Jiang LL, Li GD, Guo J, Zhang YH** 2004 [Diagnostic value of A103 and inhibin-α in adrenocortical tumors: an immunohistochemical study using tissue microarray techniques]. Zhonghua Bing Li Xue Za Zhi 33:203–207 (Chinese)
21. **Wick MR, Cherwitz DL, McGlennen RC, Dehner LP** 1986 Adrenocortical carcinoma. An immunohistochemical comparison with renal cell carcinoma. Am J Pathol 122:343–352
22. **Saeger W** 2000 Histopathological classification of adrenal tumours. Eur J Clin Invest 30(Suppl 3):58–62
23. **Sasano H, Shizawa S, Suzuki T, Takayama K, Fukaya T, Morohashi K, Nagura H** 1995 Transcription factor adrenal 4 binding protein as a marker of adrenocortical malignancy. Hum Pathol 26:1154–1156
24. **Luo X, Ikeda Y, Parker KL** 1994 A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 77:481–490
25. **Crawford PA, Sadovsky Y, Milbrandt J** 1997 Nuclear receptor steroidogenic factor 1 directs embryonic stem cells toward the steroidogenic lineage. Mol Cell Biol 17:3997–4006
26. **Parker KL, Schimmer BP** 1997 Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 18: 361–377
27. **Val P, Lefrançois-Martinez AM, Veyssière G, Martinez A** 2003 SF-1 a key player in the development and differentiation of steroidogenic tissues. Nucl Recept 1:8
28. **Figueiredo BC, Cavalli LR, Pianovski MA, Lalli E, Sandrini R, Ribeiro RC, Zambetti G, DeLacerda L, Rodrigues GA, Haddad BR** 2005 Amplification of the steroidogenic factor 1 gene in childhood adrenocortical tumors. J Clin Endocrinol Metab 90:615–619
29. **Pianovski MA, Cavalli LR, Figueiredo BC, Santos SC, Doghman M, Ribeiro RC, Oliveira AG, Michalkiewicz E, Rodrigues GA, Zambetti G, Haddad BR, Lalli E** 2006 SF-1 overexpression in childhood adrenocortical tumours. Eur J Cancer 42:1040–1043
30. **Doghman M, Arhatte M, Thibout H, Rodrigues G, De Moura J, Grosso S, West AN, Laurent M, Mas JC, Bongain A, Zambetti GP, Figueiredo BC, Auberger P, Martinerie C, Lalli E** 2007 Nephroblastoma overexpressed/cysteine-rich protein 61/connective tissue growth factor/nephroblastoma overexpressed gene-3 (NOV/CCN3), a selective adrenocortical cell proapoptotic factor, is downregulated in childhood adrenocortical tumors. J Clin Endocrinol Metab 92:3253–3260
31. **Doghman M, Karpova T, Rodrigues GA, Arhatte M, De Moura J, Cavalli LR, Virolle V, Barbry P, Zambetti GP, Figueiredo BC, Heckert LL, Lalli E** 2007 Increased steroidogenic factor-1 dosage triggers adrenocortical cell proliferation and cancer. Mol Endocrinol 21:2968–2987
32. **Lichtenauer UD, Duchniewicz M, Kolanczyk M, Hoeflich A, Hahner S, Else T, Bicknell AB, Zemojtel T, Stallings NR, Schulte DM, Kamps MP, Hammer GD, Scheele JS, Beuschlein F** 2007 Pre-B-cell transcription factor 1 and steroidogenic factor 1 synergistically regulate adrenocortical growth and steroidogenesis. Endocrinology 148:693–704
33. **Doghman M, Cazareth J, Douguet D, Madoux F, Hodder P, Lalli E** 2009 Inhibition of adrenocortical carcinoma cell proliferation by steroidogenic factor-1 inverse agonists. J Clin Endocrinol Metab 94:2178–2183
34. **Sasano H, Shizawa S, Nagura H** 1995 Adrenocortical cytopathology. Am J Clin Pathol 104:161–166
35. **Sasano H, Shizawa S, Suzuki T, Takayama K, Fukaya T, Morohashi K, Nagura H** 1995 Ad4BP in the human adrenal cortex and its disorders. J Clin Endocrinol Metab 80:2378–2380
36. **Kaneko T, Kojima Y, Umemoto Y, Sasaki S, Hayashi Y, Kohri K** 2008 Usefulness of transcription factors Ad4BP/SF-1 and DAX-1 as immunohistologic markers for diagnosis of advanced adrenocortical carcinoma. Horm Res 70:294–299
37. **Koschker AC, Fassnacht M, Hahner S, Weismann D, Allolio B** 2006 Adrenocortical carcinoma: improving patient care by establishing new structures. Exp Clin Endocrinol Diabetes 114:45–51
38. **Mansmann G, Lau J, Balk E, Rothberg M, Miyachi Y, Bornstein SR** 2004 The clinically inapparent adrenal mass: update in diagnosis and management. Endocr Rev 25:309–340
39. **de Reyniès A, Assié G, Rickman DS, Tissier F, Groussin L, René-Corail F, Dousset B, Bertagna X, Clauser E, Bertherat J** 2009 Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. J Clin Oncol 27:1108–1115
40. **Abiven G, Coste J, Groussin L, Anract P, Tissier F, Legmann P, Dousset B, Bertagna X, Bertherat J** 2006 Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients. J Clin Endocrinol Metab 91:2650–2655
41. **Bertherat J, Contesse V, Louiset E, Barrande G, Duparc C, Groussin**

L, Emy P, Bertagna X, Kuhn JM, Vaudry H, Lefebvre H 2005 *In vivo* and *in vitro* screening for illegitimate receptors in adrenocorticotropin-independent macronodular adrenal hyperplasia causing Cushing's syndrome: identification of two cases of gonadotropin/gastric inhibitory polypeptide-dependent hypercortisolism. J Clin Endocrinol Metab 90:1302–1310

42. **Weismann D, Briese J, Niemann J, Grüneberger M, Adam P, Hahner S, Johanssen S, Liu W, Ezzat S, Saeger W, Bamberger AM, Fassnacht M, Schulte HM, Asa SL, Allolio B, Bamberger CM** 2009 Osteopontin stimulates invasion of NCI-h295 cells but is not associated with survival in adrenocortical carcinoma. J Pathol 218:232–240
43. **Ronchi CL, Sbiera S, Kraus L, Wortmann S, Johanssen S, Adam P, Willenberg HS, Hahner S, Allolio B, Fassnacht M** 2009 Expression of excision repair cross complementing group 1 and prognosis in adrenocortical carcinoma patients treated with platinum-based chemotherapy. Endocr Relat Cancer 16:907–918
44. **Fenske W, Völker HU, Adam P, Hahner S, Johanssen S, Wortmann S, Schmidt M, Morcos M, Müller-Hermelink HK, Allolio B, Fassnacht M** 2009 Glucose transporter GLUT1 expression is an stage-independent predictor of clinical outcome in adrenocortical carcinoma. Endocr Relat Cancer 16:919–928
45. **Olaussen KA, Dunant A, Fouret P, Brambilla E, André F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC** 2006 DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 355:983–991
46. **Landis JR, Koch GG** 1977 The measurement of observer agreement for categorical data. Biometrics 33:159–174
47. **Coenraads PJ, Van Der Walle H, Thestrup-Pedersen K, Ruzicka T, Dreno B, De La Loge C, Viala M, Querner S, Brown T, Zultak M** 2005 Construction and validation of a photographic guide for assessing severity of chronic hand dermatitis. Br J Dermatol 152:296–301
48. **Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP** 2003 Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 31:e15
49. **Fassnacht M, Johanssen S, Quinkler M, Bucsky P, Willenberg HS, Beuschlein F, Terzolo M, Mueller HH, Hahner S, Allolio B** 2009 Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification. Cancer 115:243–250
50. **Shibata H, Ikeda Y, Mukai T, Morohashi K, Kurihara I, Ando T, Suzuki T, Kobayashi S, Murai M, Saito I, Saruta T** 2001 Expression profiles of COUP-TF, DAX-1, and SF-1 in the human adrenal gland and adrenocortical tumors: possible implications in steroidogenesis. Mol Genet Metab 74:206–216
51. **Volante M, Buttigliero C, Greco E, Berruti A, Papotti M** 2008 Pathological and molecular features of adrenocortical carcinoma: an update. J Clin Pathol 61:787–793
52. **Saeger W, Fassnacht M, Chita R, Prager G, Nies C, Lorenz K, Bärlehner E, Simon D, Niederle B, Beuschlein F, Allolio B, Reincke M** 2003 High diagnostic accuracy of adrenal core biopsy: results of the German and Austrian adrenal network multicenter trial in 220 consecutive patients. Hum Pathol 34:180–186
53. **Kojima Y, Sasaki S, Hayashi Y, Umemoto Y, Morohashi K, Kohri K** 2006 Role of transcription factors Ad4bp/SF-1 and DAX-1 in steroidogenesis and spermatogenesis in human testicular development and idiopathic azoospermia. Int J Urol 13:785–793
54. **Sato Y, Terada Y, Utsunomiya H, Koyanagi Y, Ito M, Miyoshi I, Suzuki T, Sasano H, Murakami T, Yaegashi N, Okamura K** 2003 Immunohistochemical localization of steroidogenic enzymes in human follicle following xenotransplantation of the human ovarian cortex into NOD-SCID mice. Mol Reprod Dev 65:67–72
55. **Takayama K, Sasano H, Fukaya T, Morohashi K, Suzuki T, Tamura M, Costa MJ, Yajima A** 1995 Immunohistochemical localization of Ad4-binding protein with correlation to steroidogenic enzyme expression in cycling human ovaries and sex cord stromal tumors. J Clin Endocrinol Metab 80:2815–2821
56. **Dohna M, Reincke M, Mincheva A, Allolio B, Solinas-Toldo S, Lichter P** 2000 Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. Genes Chromosomes Cancer 28:145–152
57. **Almeida MQ, Soares IC, Ribeiro TC, Fragoso MC, Marins LV, Wakamatsu A, Ressio RA, Nishi MY, Jorge AA, Lerario AM, Alves VA, Mendonca BB, Latronico AC** 2010 Steroidogenic factor 1 overexpression and gene amplification are more frequent in adrenocortical tumors from children than from adults. J Clin Endocrinol Metab 95:1458–1462
58. **Weber MM, Fottner C, Wolf E** 2000 The role of the insulin-like growth factor system in adrenocortical tumourigenesis. Eur J Clin Invest 30(Suppl 3):69–75
59. **Ilvesmäki V, Blum WF, Voutilainen R** 1993 Insulin-like growth factor-II in human fetal adrenals: regulation by ACTH, protein kinase C and growth factors. J Endocrinol 137:533–542
60. **de Fraipont F, El Atifi M, Cherradi N, Le Moigne G, Defaye G, Houlgatte R, Bertherat J, Bertagna X, Plouin PF, Baudin E, Berger F, Gicquel C, Chabre O, Feige JJ** 2005 Gene expression profiling of human adrenocortical tumors using complementary deoxyribonucleic acid microarrays identifies several candidate genes as markers of malignancy. J Clin Endocrinol Metab 90:1819–1829
61. **Giordano TJ, Kuick R, Else T, Gauger PG, Vinco M, Bauersfeld J, Sanders D, Thomas DG, Doherty G, Hammer G** 2009 Molecular classification and prognostication of adrenocortical tumors by transcriptome profiling. Clin Cancer Res 15:668–676
62. **Dlugosz AA, Talpaz M** 2009 Following the hedgehog to new cancer therapies. N Engl J Med 361:1202–1205
63. **Assié G, Antoni G, Tissier F, Caillou B, Abiven G, Gicquel C, Leboulleux S, Travagli JP, Dromain C, Bertagna X, Bertherat J, Schlumberger M, Baudin E** 2007 Prognostic parameters of metastatic adrenocortical carcinoma. J Clin Endocrinol Metab 92:148–154
64. **Stojadinovic A, Ghossein RA, Hoos A, Nissan A, Marshall D, Dudas M, Cordon-Cardo C, Jaques DP, Brennan MF** 2002 Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. J Clin Oncol 20:941–950
65. **Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E, Reimondo G, Bollito E, Papotti M, Saeger W, Hahner S, Koschker AC, Arvat E, Ambrosi B, Loli P, Lombardi G, Mannelli M, Bruzzi P, Mantero F, Allolio B, Dogliotti L, Berruti A** 2007 Adjuvant mitotane treatment for adrenocortical carcinoma. N Engl J Med 356:2372–2380
66. **Fassnacht M, Hahner S, Polat B, Koschker AC, Kenn W, Flentje M, Allolio B** 2006 Efficacy of adjuvant radiotherapy of the tumor bed on local recurrence of adrenocortical carcinoma. J Clin Endocrinol Metab 91:4501–4504
67. **Huang H, Fojo T** 2008 Adjuvant mitotane for adrenocortical cancer–a recurring controversy. J Clin Endocrinol Metab 93:3730–3732
68. **Del Tredici AL, Andersen CB, Currier EA, Ohrmund SR, Fairbain LC, Lund BW, Nash N, Olsson R, Piu F** 2008 Identification of the first synthetic steroidogenic factor 1 inverse agonists: pharmacological modulation of steroidogenic enzymes. Mol Pharmacol 73:900–908
69. **Madoux F, Li X, Chase P, Zastrow G, Cameron MD, Conkright JJ, Griffin PR, Thacher S, Hodder P** 2008 Potent, selective and cell penetrant inhibitors of SF-1 by functional ultra-high-throughput screening. Mol Pharmacol 73:1776–1784
70. **Roth J, Madoux F, Hodder P, Roush WR** 2008 Synthesis of small molecule inhibitors of the orphan nuclear receptor steroidogenic factor-1 (NR5A1) based on isoquinolinone scaffolds. Bioorg Med Chem Lett 18:2628–2632