Adrenocortical Carcinoma

An Immunohistochemical Comparison With Renal Cell Carcinoma

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The diagnosis of adrenocortical carcinoma (ACC) is often difficult, because this tumor may present with direct extension into adjacent renal parenchyma or with metastatic disease. Renal cell carcinoma and other histologically similar tumors are potentially confused with ACC by conventional light microscopy, and their separation from the latter is often impossible without the aid of additional studies. Furthermore, the distinction between adrenal cortical adenoma and ACC may also be problematic. Because of these factors, the authors studied 10 cases each of ACC, adrenocortical adenoma, and renal cell carcinoma (RCC) immunohistochemically, in an attempt to develop objective parameters which may aid in this differential diagnostic dilemma. Nontrypsinized, formalin-fixed, paraffin-embedded specimens were used in all cases, and tissue from the adrenocortical tumors was also studied for intermediate filament content after protease digestion. All 20 nontrypsinized adrenocortical neoplasms were positive for vimentin, but not for cytokeratin, epithelial membrane antigen, or blood group isoantigens. Conversely, each of 10 cases of RCC expressed epithelial membrane antigen, cytokeratin, and blood group isoantigens, but none was immunoreactive for vimentin. Two adenocortical carcinomas and three adenomas manifested cytokeratin positivity after trypsin digestion. There were no significant differences between the immunostaining profiles of ACC and adrenocortical adenoma, which suggest that this distinction must still rely upon clinical and morphologic criteria. (Am J Pathol 1986, 122:343–352)

IN THE ABSENCE of clinical signs of adrenal cortical hyperfunction such as Cushing's syndrome or inappropriate masculinization or feminization, the preoperative diagnosis of adrenocortical carcinoma (ACC) may present considerable difficulties. The specimen in question may represent a metastatic nodule or a lymph node containing trabecular profiles, nests, or formless sheets of large epithelioid cells, with granular, eosinophilic, or clear cytoplasm. In these circumstances, the differential diagnosis includes not only ACC but renal cell carcinoma (RCC), hepatocellular carcinoma, malignant melanoma, and anaplastic carcinoma of the lung and other sites.[1] Because of the juxtaposition of the adrenal gland to the kidney, it is not uncommon for ACC to involve the renal parenchyma at diagnosis. Also, RCC may occasionally present as a metastasis to the contralateral adrenal gland.[2] The consequences of the latter situation may include an inappropriate adrenalectomy/nephrectomy for what is erroneously regarded as an ACC.

Another problem with respect to adrenocortical neoplasms, even in the presence of endocrine dysfunction, is the pathologic distinction between benign and malignant tumors. Criteria derived from multivariate analysis of the gross and microscopic features of clinically verified adenomas and carcinomas[3–8] still do not allow this separation to be made in all cases.

With these difficulties in mind, we compared the immunohistochemical profiles of 10 cases of clinically confirmed ACC with those of RCC and adrenocortical adenoma. In this report, the results of this analysis are presented.

Materials and Methods

The files of the Division of Surgical Pathology at the University of Minnesota were surveyed for examples of ACC, seen between 1960 and 1984. Retrieved cases were

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Table 1—Immunohistologic Reagents Used in the Study of Adrenocortical Carcinoma, Adrenocortical Adenoma, and Renal Cell Carcinoma

Reagent	Source	Dilution
Anti-cytokeratins		
44, 46, 52, 54 kd—clone designation PKK1	Lab Systems, Inc.	1:160
54 kd—clone designation 35BH11	Enzo Biochem, Inc.	1:4000
49, 51, 57, 66 kd—clone designation 34BE12	Enzo Biochem, Inc.	1:4000
40, 50, 56.5, 58, 65–67 kd—clone designations AE1/AE3	Hybritech, Inc.	1:200
Anti-vimentin		
Clone designation PK-V	Lab Systems, Inc.	1:40
Single immunoblot 58 kd	Dr. Susan Simonton	1:80
Clone designation BM52	Department of Pathology University of Minnesota	
Anti-carcinoembryonic antigen	Hybritech, Inc.	1:160
Anti-epithelial membrane antigen	DakoPatts Co., Inc.	1:160
Anti-S100 protein	DakoPatts Co., Inc.	1:1600
Anti-alphafetoprotein	DakoPatts Co., Inc.	1:700
Anti-α_1 antitrypsin	DakoPatts Co., Inc.	1:320
Anti-β_2 microglobulin	Dr. Russell Curry, Department of Medicine, Univeristy of Minnesota	1:300
Anti-blood group isoantigens A, B, and H	DakoPatts, Inc.	1:80
Biotinylated peanut agglutinin	Vector Laboratories	1:1600
Biotinylated wheat germ agglutinin	Vector Laboratories	1:3200
Biotinylated soybean agglutinin	Vector Laboratories	1:4000
Biotinylated *Dolichos biflorus* agglutinin	Vector Laboratories	1:3200
Biotinylated *Ulex europaeus* lectin	Vector Laboratories	1:3600
Sheep antirabbit globulin	Antibodies, Inc.	1:75
Horse antimouse globulin (biotinylated)	Vector Laboratories	—*
Rabbit peroxidase–antiperoxidase complex	Sternberger Laboratories	1:300
Avidin–biotin–peroxidase complex	Vector Laboratories	—*

* Vectastain Kit (Mouse).

reexamined histologically, and clinical data on them were reviewed. Only those neoplasms which behaved in a malignant fashion were retained in the study group (10 cases). For comparison, 10 cases of adrenocortical adenoma were selected for study, along with 10 randomly chosen examples of RCC.

Formalin-fixed, paraffin-embedded tissue from these 30 cases was stained with the use of the peroxidase-antiperoxidase (PAP) or avidin-biotin-peroxidase complex (ABC) techniques, as previously described.[9,10] Sections were deparaffinized in xylene, incubated in methanolic hydrogen peroxide solution (0.6%) for 30 minutes, and rehydrated in graded alcohols and distilled water. After incubation in phosphate-buffered saline (PBS, pH 7.5) for 10 minutes, primary antibodies were applied, and sections were then incubated in moisture chambers for 18 hours at 4 C. Prior protease digestion was not employed in this portion of the study.

After rinsing with PBS, biotinylated horse anti-mouse or sheep anti-rabbit globulins were applied (for monoclonal and polyclonal primary antibodies, respectively), followed by incubation at room temperature for 1 hour. Sections were again rinsed in PBS and incubated with ABC or rabbit PAP complex for 1 hour at room temperature. Sections to which lectins had been applied were incubated with ABC for 1 hour at room temperature, after rinsing in PBS. Following a final rinse in phosphate buffer (pH 7.4), chromogenic development was accomplished by immersion of sections in phosphate buffer–3,3′-diaminobenzidine solution (0.025–0.25 mg/ml) with 0.01% hydrogen peroxide, for 10 minutes. They were counterstained with Harris' hematoxylin, dehyrated, coverslipped with Permount, and examined by conventional light microscopy.

In addition, sections from each of the 20 adrenocortical tumors were exposed to 0.1% bovine trypsin (Sigma Chemical Co.) in 0.01% HCl/PBS, for 20 minutes at room temperature, before application of anti-cytokeratin and anti-vimentin antibodies. The remainder of the immunohistochemical ABC technique was carried out as specified above.

Primary antibodies utilized in these procedures included monoclonal anti-cytokeratin (hydridoma clones PKK1, AE1/AE3, 35BH11, and 34BE12),[11–13] anti-vimentin (hybridoma clones PK-V and BM-52),[14] anti-carcinomembryonic antigen (CEA), -epithelial membrane antigen (EMA), and anti-blood group isoantigens A, B, and H (BGI). Polyclonal rabbit antibodies to S100 protein, α-fetoprotein (AFP), α-antitrypsin (AAT), and β_2-microglobulin (B2M) were also employed. In addition, biotinylated peanut agglutinin (PNA), wheat germ agglutinin (WGA), soybean agglutinin (SBA), *Dolichos*

Table 2—Clinical Data on Adrenocortical Carcinoma, Adrenocortical Adenoma, and Renal Cell Carcinoma

Case	Age/sex	Tumor size/weight	Clinical endocrinopathy?	Treatment	Outcome
			Adrenocortical carcinoma		
1	55/F	10.5 cm GD /NR	0	None	Died 1 month after diagnosis with metastases to heart, lung, bone marrow, pancreas, and brain.
2	80/F	Biopsy only	+: Cushing's	None	Died 1 month after diagnosis with metastases to liver
3	67/F	Biopsy only	+: Cushing's	None	Died 2 weeks after diagnosis with metastases to liver and lungs
4	29/F	16 cm GD/ 675 g	+: Cushing's	S + C	Metastases to lungs 4 years after diagnosis, surgically resected; patient free of disease 8 years after diagnosis
5	20/M	14 cm GD/ 1200 g	0*	S + C	Alive with metastases to lungs and liver, 18 months after diagnosis
6	62/M	12 cm GD/ NR	+: Conn's	S + C	Alive with metastases to lungs, 4 years after diagnosis
7	36/M	10 cm GD/ NR	0	S	Died 4 months after diagnosis with metastases to bones, lungs, and soft tissue
8	55/M	NR/NR	0	S + R + C	Alive 10 years after diagnosis; local recurrence 8 years after diagnosis (resected): metastases to lungs 10 years after diagnosis
9	66/M	15 cm GD/ 500 g	0	S	Alive with metastases to lungs, 3 years after diagnosis
10	30/M	NR/NR	0	S + C	Pulmonary metastasis at diagnosis (resected); alive 3 months after diagnosis

Case	Age/sex	Tumor size/weight	Clinical endocrinopathy?	Treatment	Outcome
			Adrenocortical adenomas		
11	9 mo/M	7 cm GD/ 200 g	+: Cushing's	S	NED: 3 years
12	71/F	4 cm GD/ 50 g	0	S	NED: 3 years
13	68/F	NR/NR	0	S	NED: 2.5 years
14	19/F	3 cm GD/ NR	+: Cushing's	S	NED: 2 years
15	46/M	1.8 cm GD/ NR	+: Conn's	S	NED: 4 years
16	60/F	2.5 cm GD/ NR	+: Cushing's	S	NED: 6 years
17	49/F	1.6 cm GD/ NR	+: Conn's	S	NED: 5 years
18	3/F	6.5 cm GD/ NR	+: Conn's	S	NED: 5 years
19	27/F	5 cm GD/ 30.5 g	+: Cushing's	S	NED: 5 years
20	39/F	2 cm GD/ 6 g	+: Conn's	S	NED: 2 years

Case	Age/sex	Clinical paraneoplasia?	Treatment	Metastases?	Outcome
			Renal cell carcinomas		
21	73/M	0	S	0	DOC: 0.6 years
22	56/F	+: Hypertension	S	+: RLN	NED: 6.2 years
23	73/F	0	S	+: Lungs, bones	NED: 6 years
24	60/M	? (Adrenal hyperplasia)	S	+: Lungs	DOD: 0.7 years
25	60/F	0	S	+: Lungs, bones	DOD: 0.7 years
26	61/F	0	S	+: Lungs, bone, brain	DOD: 0.2 years
27	70/M	0	S	?	DUC: 0.9 years
28	57/M	0	S	0	NED: 5 years
29	46/F	0	S	+: RLN	NED: 5 years
30	57/M	0	S	0	NED: 4, 5 years

GD, greatest dimension; NR, not recorded; S, surgery; R, radiotherapy; C, chemotherapy; NED, no evidence of disease; DOC, dead of other causes, DOD, dead of disease (tumor); DUC; dead of unknown cause; RLN, regional lymph nodes.

* Elevated level of urinary 17-ketosteroids.

biflorus lectin (DBL), and *Ulex europaeus* lectin (UEL) were used in histochemical analyses of the 30 tumors. The sources and working dilutions for these reagents are listed in Table 1.

Positive controls were represented by sections of stock tissues and neoplasms known to contain the antigens being studied. Negative control sections of the 30 neoplasms were obtained by substituting nonimmune rabbit serum or murine ascites fluid for primary antibodies, in the PAP and ABC methods, respectively.

Results

Clinical Findings

The clinical aspects of each case are summarized in Table 2.

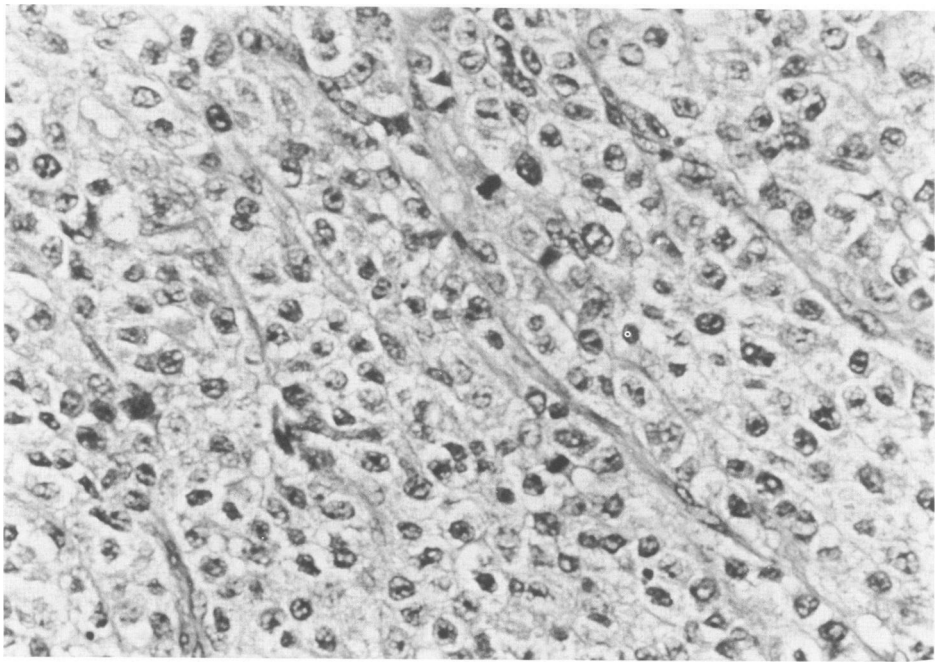

Figure 1—Adrenocortical carcinoma, composed of cells with clear or slightly granular cytoplasm, with minimal nuclear pleomorphism. Mitotic figures are evident. (H&E, ×250)

Pathologic Findings

Macroscopic Features

The gross features in cases of resected ACC were typical of this diagnosis.[3-6,8] The native adrenal gland usually was not apparent within such masses. The sizes of ACC averaged 13 cm in greatest dimension, and the mean weight in three examples was 792 g.

In contrast, adrenocortical adenomas were well-circumscribed and were bordered by easily identifiable adrenal tissue. They were brown or yellow, without hemorrhage or necrosis, and measured 3.1 cm in average diameter; the largest weighed 200 g.

All 10 RCCs had a classic gross appearance. None involved the adrenal glands.

Microscopic Observations

Adrenocortical Carcinomas

Variable cytologic appearances characterized the cases of ACC in this series. Four tumors were composed of round to polygonal cells, with round or slightly irregular nuclei and indistinct nucleoli. The cytoplasm was abundantly vacuolated in these tumors, which gave them a "clear-cell" appearance (Figure 1). Another 4 cases had smaller cells with granular eosinophilic cytoplasm (Figure 2). Two further cases were composed of sheets of poorly cohesive, polygonal, or slightly spindled cells, with vesicular nuclei, prominent nucleoli, and scant amphophilic cytoplasm.

Additional features included extensive necrosis, occasional "giant" tumor cells, and mitotic rates ranging from 1–3 per high-power (400×) field, with abnormal mitoses in 8 cases. Vascular invasion was observed in 4 cases, and capsular invasion was seen in 5.

Adrenocortical Adenomas

Nine adenomas were typified by nests and cords of cohesive, vacuolated tumor cells, with round to oval nuclei, abundant cytoplasm, and distinct borders. Mitoses were lacking, but focal nuclear hyperchromasia and atypia were seen in 3 cases. None displayed nucleolar prominence, inflammation, necrosis, capsular permeation, or vascular invasion.

Renal Cell Carcinomas

All 10 renal cell carcinomas included in this study had a clear-cell cytologic appearance (Figure 3). Five were Grade 2 neoplasms, and 5 were Grade 3, according to the criteria of Fuhrman et al.[15] These tumors displayed clustered or medullary cellular growth patterns, with focal necrosis and hemorrhage. Mitoses averaged one to two per high-power field.

Immunohistochemical Findings (Table 3)

All 20 adrenocortical tumors, whether benign or malignant, stained positively for vimentin content in a diffuse cytoplasmic pattern, both with and without prior trypsinization (Figure 4). Nontrypsinized specimens of these neoplasms were uniformly negative for cytokeratin, EMA, AAT, AFP, S100 protein, CEA, BGI,

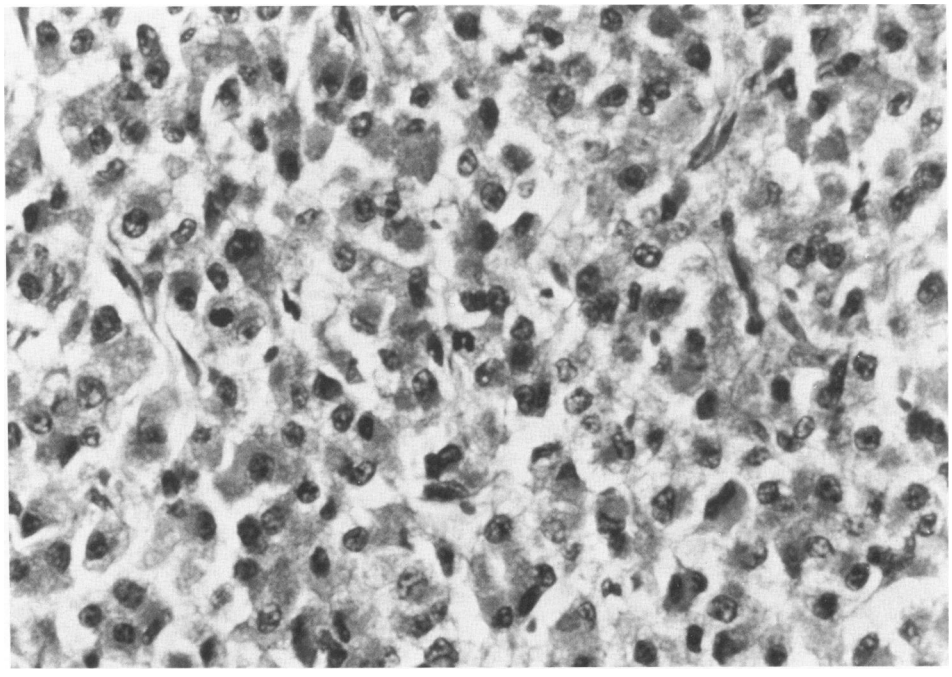

Figure 2—Adrenocortical carcinoma made up of smaller cells than those seen in Figure 1, with more granular cytoplasm. (H&E, ×400)

and UEL and DBA binding. Two ACCs bound PNA, 3 bound SBA, and 1 bound WGA. Two displayed membrane positivity for B2M.

In addition to vimentin, B2M was the only other antigen expressed by nontrypsinized adrenocortical adenomas (3 cases) (Figure 5). The nonneoplastic adrenal cortical tissue included in specimens of adenoma showed identical antigenic profiles.

Two trypsinized adrenocortical carcinomas showed reactivity with anti-cytokeratins AE1/AE3 and PKKl; 3 similarly treated adrenocortical adenomas and 5 specimens of adenoma-related normal adrenal cortex showed comparable positivity. None reacted with 35BHll or 34BE12.

Nontrypsinized renal cell carcinomas uniformly expressed EMA (Figure 6) and cytokeratin. All displayed

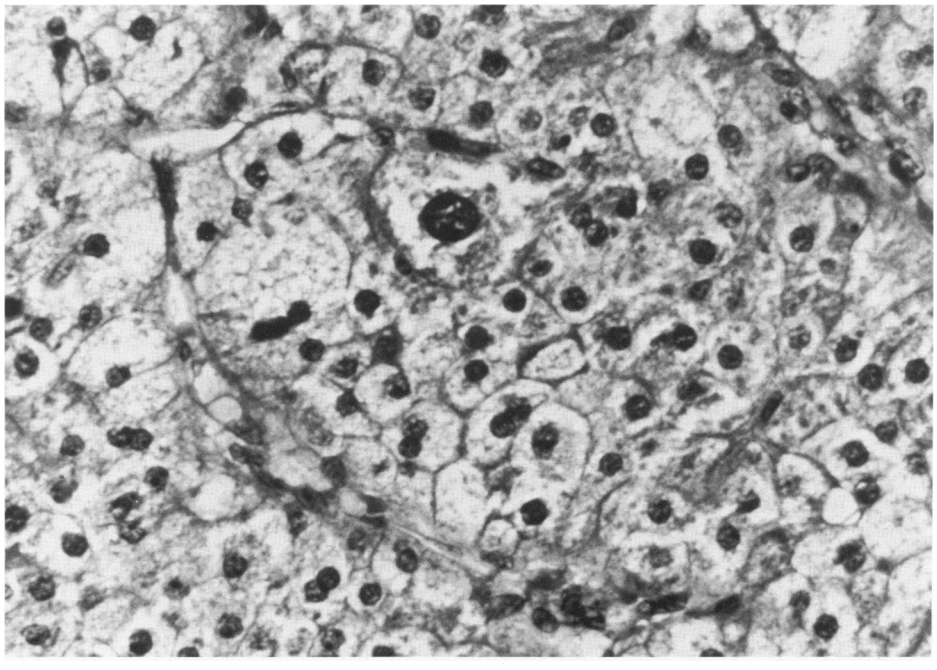

Figure 3—Typical renal cell carcinoma, composed of cells with slight to moderate nuclear atypia, and clear cytoplasm. (H&E, ×400)

Table 3—Immunostaining Results in Adrenocortical Carcinoma, Adrenocortical Adenoma, and Renal Cell Carcinoma*

Antigen/lectin	ACC	ACA	RCC
Cytokeratins			
AE1/AE3	0/10†‡	0/10‡	10/10
PKK1	0/10‡	0/10‡	7/10 (Cases 21, 22, 23, 26, 27, 28, 29)
35BH11	0/10	0/10	2/10 (Cases 26, 28)
34BE12	0/10	0/10	2/10 (Cases 26, 28)
Vimentin			
PK-V	10/10	10/10	0/10
BM-52	10/10	10/10	0/10
Carcinoembryonic antigen	0/10	0/10	0/10
Epithelial membrane antigen	0/10	0/10	10/10
S100 Protein	0/10	0/10	0/10
α-fetoprotein	0/10	0/10	0/10
α_1-antitrypsin	0/10	0/10	0/10
β_2-microglobulin	2/10 (Cases 2, 8)	3/10 (Cases 12, 15, 17)	7/10 (Cases 22–25, 28–30)
Blood group isoantigens	0/10	0/10	10/10
Peanut agglutinin	2/10 (Cases 1.5)	0/10	7/10 (Cases 21–26, 30)
Wheat germ agglutinin	1/10 (Case 1)	0/10	6/10 (Cases 21, 22, 24–27)
Soybean agglutinin	3/10 (Cases 1, 4, 5)	0/10	2/10 (Cases 21, 26)
Dolichos biflorus lectin	0/10	0/10	5/10 (Cases 21–23, 28, 29)
Ulex europaeus lectin	0/10	0/10	0/10

ACC, adrenocortical carcinoma; ACA, adrenocortical adenoma; RCC, renal cell carcinoma.
* All results refer to exclusive use of nontrypsinized specimens.
† Cases positive/cases studied.
‡ Cases 2, 4, 15, 17, and 18 were positive with these antisera, after trypsin digestion.

reactivity with AE1/AE3, 7 were positive with PKKl, and 2 reacted with 35BHll and 34BE12. Seven tumors manifested B2M positivity. Nine bound PNA, 2 bound SBA, 6 bound WGA, and 5 bound DBA. None displayed binding of UEL. All 10 renal cell carcinomas expressed immunoreactivity for BGI (Figure 7), in a mixed membranous and cytoplasmic pattern. In contrast, all failed to stain for CEA, AFP, AAT, or S100 protein content.

Positive and negative controls stained appropriately.

Discussion

Adrenocortical carcinoma is an uncommon neoplasm, with a reported incidence of 2 cases per million individuals per year.[16] Hence, when ACC presents with metastasis, pathologists are confronted with a relatively unfamiliar tumor in terms of its morphologic recognition. This problem is compounded by the overlapping histologic features of ACC, RCC, malignant melanoma, hepatocellular carcinoma, and anaplastic carci-

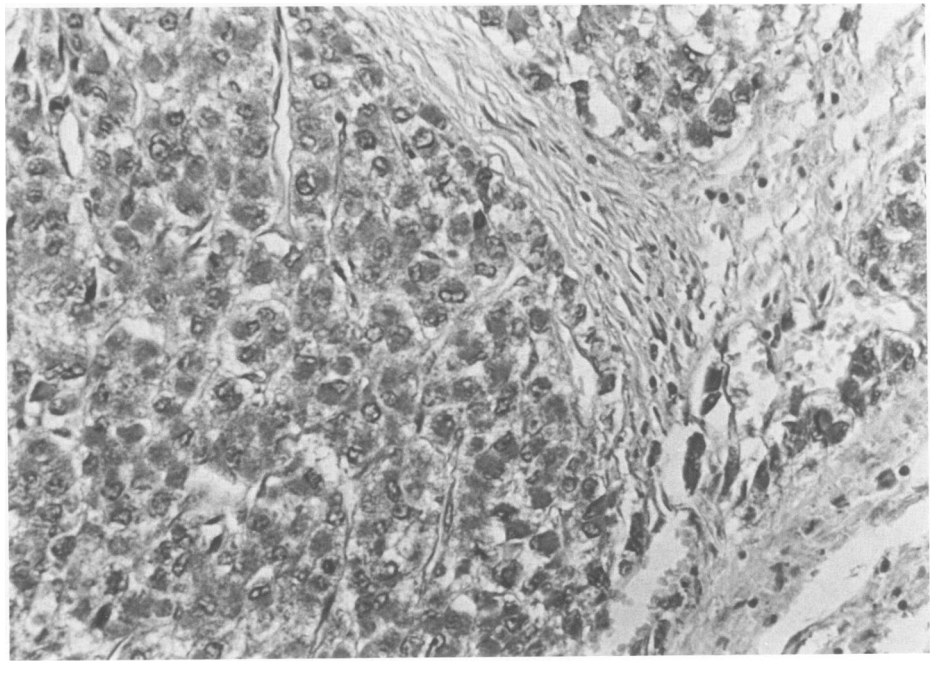

Figure 4—Immunohistochemical staining for vimentin in adrenocortical carcinoma. Tumor cells show diffuse cytoplasmic reactivity. (Anti-vimentin/ABC stain with light hematoxylin counterstain, ×250)

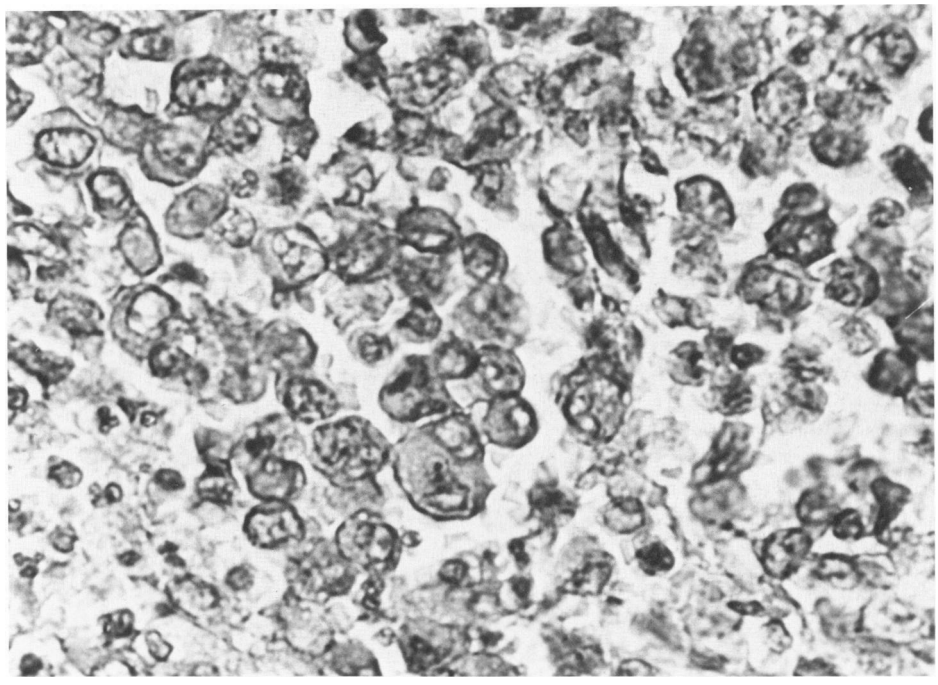

Figure 5—Membrane staining for β_2-microglobulin in adrenocortical adenoma, represented by a dark rim around individual tumor cells (Anti-B2M/PAP stain with light hematoxylin counterstain, ×400)

noma of the lung, as mentioned previously. These neoplasms may have metastasized to the adrenal glands at diagnosis, and further confusion therefore results as to which organ is the primary site.

Of these differential diagnostic possibilities, RCC is usually the most troublesome entity to exclude, by conventional histologic study. If electron-microscopic specimens are available, ultrastructural study can contribute to the separation of this tumor from ACC in some cases,[1] but we elected to focus our attention on an immunohistochemical comparison between RCC and ACC, because this modality of study is more widely available and less expensive. The results of this analysis indicate that these two tumors are indeed separable by such means. All 10 ACCs were vimentin-positive and EMA- and BGI-negative, while the converse of these findings was obtained in an equal number of renal cell carcinomas. In addition, all cases of RCC contained cytokeratin, whereas none of the adrenocortical carcinomas manifested reactivity for this intermediate fila-

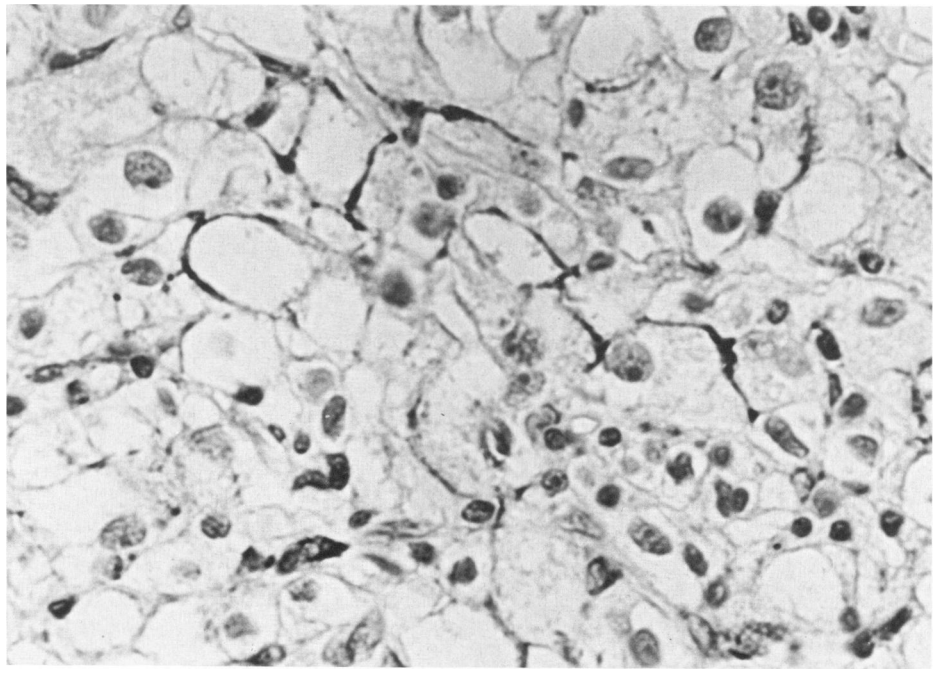

Figure 6—Positive immunostaining for epithelial membrane antigen in renal cell carcinoma, represented by dark membrane-based deposition of reaction product. (Anti-EMA/ABC stain with light hematoxylin counterstain, ×400)

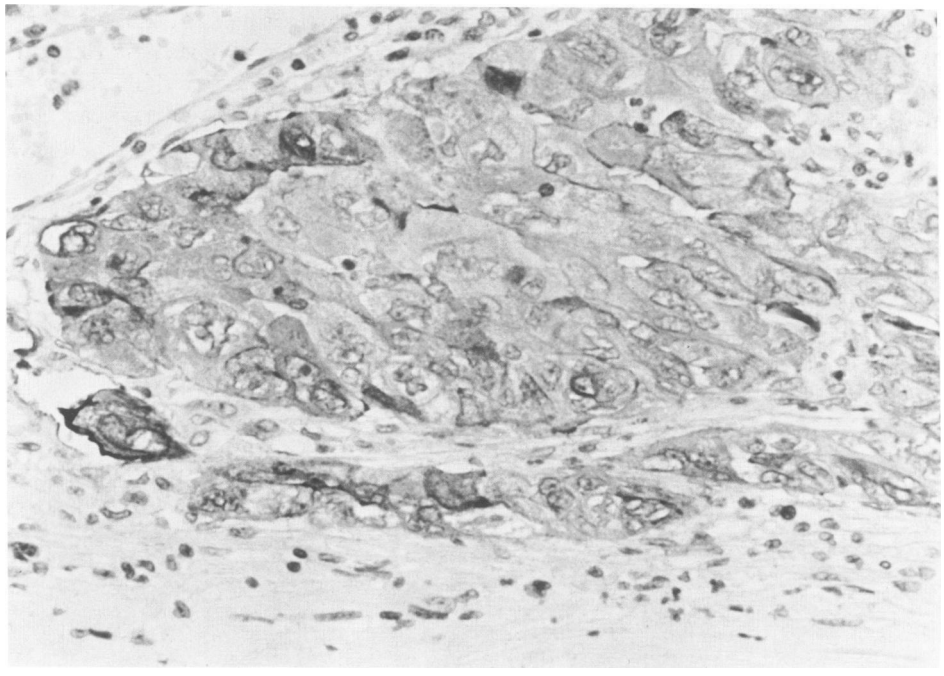

Figure 7—Immunoreactivity for blood group isoantigen A in renal cell carcinoma, with both membrane-based and cytoplasmic staining. (Anti-BGI-A/ABC stain, with light hematoxylin counterstain, × 400)

ment with one of the methods we employed (lack of trypsin digestion).

Procedural dissimilarities account for the differences between some intermediate filament reactivities seen in our study and those observed by Miettinen et al[17] in adrenal tissues and tumors. These authors noted the presence of cytokeratin in most of the adrenocortical tumors which were analyzed, but they uniformly employed trypsin digestion of formalin-fixed tissue, as well as specimens fixed in absolute alcohol, and frozen sections. These factors are known to enhance cytokeratin reactivity, compared with that seen in unmodified formalin-fixed tissue, as also suggested by our results in trypsinized tumors.[18] Indeed, it is likely that they are *necessary* to demonstrate keratin proteins in formalin-fixed adrenal tumors, perhaps because of low antigen density in such lesions. On the other hand, RCC appears to express cytokeratin positivity even in nontrypsinized specimens, especially with selected monoclonal antibodies (AE1/AE3 and PKK1). Hence, even though it does not maximize cytokeratin detection, the omission of protease digestion of formalin-fixed tissue contributes to a diagnostically useful means of separating ACC and RCC, in concert with immunostaining for EMA and BGI. Sun et al[19] and Nagle and colleagues[20] have also observed a lack of cytokeratin reactivity in adrenocortical neoplasms, using nontrypsinized tissue.

The absence of EMA observed in adrenal cortex and adrenocortical tumors is in accord with the findings of Sloane and Ormerod.[21] Although the adrenal is conceptualized as an "epithelial" structure, paraffinized specimens of this gland and its tumors appear to lack this membrane antigen, which is expressed by most other epithelia.

The observation of vimentin in ACC also has literary precedent. Miettinen et al[17] found this intermediate filament in 8 of 21 formalin-fixed adrenocortical tumors. Although we did not detect vimentin positiv-

Table 4—Immunohistochemical Findings in Adrenocortical Carcinoma and Its Differential Diagnostic Alternatives*

Tumor	CKER	EMA	VIM	AFP/AAT	CEA	S100	BGI
Adrenocortical carcinoma	0	0	+	0	0	0	0
Renal cell carcinoma	+	+	±	0	0	0	+
Hepatocellular carcinoma	+	0	0	+ (75–92%)	+ (30%)	0	NS
Anaplastic carcinomas of lung	+	+	0	NS	+	0	±
Malignant melanoma	0	0	+	NS	0	+	0

CKER, cytokeratin; EMA, epithelial membrane antigen; AFP/AAT, α-fetoprotein/α_1-antitrypsin; CEA, carcinoembryonic antigen; S100, S100 protein; BGI, blood group isoantigens A, B, H; NS, not studied.

* Predicted results are based on the authors' experience and published data on respective tumors (see text); all results refer to use of nontrypsinized specimens.

ity in any of 10 renal cell carcinomas, Herman et al have shown that such tumors may indeed contain this protein.[22]

Results of lectin binding studies in cases of ACC and RCC showed no significant differences, negating their value in differential diagnosis of these two tumors. Similarly, further immunohistochemical studies of RCC and ACC which we have performed with monoclonal antibodies to urinary tract antigens (URO-2,3,4, Ortho Diagnostics Systems, Inc.) have shown a lack of discrimination between the two neoplasms (unpublished data). Others have made comparable observations on the specificity of these reagents.[23]

The immunohistologic features of ACC were virtually identical to those of adrenocortical adenoma and normal adrenal cortex. A loss of cell membrane-associated B2M and BGI has been linked to malignant transformation in neoplasms of the skin and the urinary tract, respectively,[24,25] but a trend of this type was not apparent in our cases of ACC and adrenocortical adenoma. Therefore, one must still rely upon the use of microscopic criteria such as those outlined by Weiss[6] and van Slooten et al[8] to discern the biologic potential of adrenocortical neoplasms. These include nuclear atypia, mitotic activity, the presence of atypical mitoses, loss of cytoplasmic clarity, a diffuse growth pattern, necrosis, and vascular or capsular invasion, as indicators of carcinomatous change. Additional factors such as overall tumor size and weight are also helpful in the evaluation of the likely clinical behavior of any given adrenocortical tumor.[26,27]

Additional antisera which were employed in this series were chosen to provide comparative, methodology-related, immunohistochemical data on ACC, with reference to hepatocellular carcinoma, malignant melanoma, and anaplastic non-small-cell lung cancer. Although a systematic intralaboratory comparison of these tumors with ACC was not performed, published accounts and our own informal observations of their immunostaining characteristics indicate that ACC is dissimilar to each of them, if one utilizes nontrypsinized tissue. Most hepatocellular carcinomas are cytokeratin-, AFP-, and AAT-positive[28,29] (11 of 12 cases studied in our laboratory); whereas ACC appears to lack all three of these substances. Malignant melanomas, like ACC, are reactive for vimentin but lack EMA and cytokeratin.[20,21,30] However, the overwhelming majority of melanomas contain S100 protein, in contrast to ACC.[31] Pulmonary large-cell carcinomas and poorly differentiated adenocarcinomas have uniformly expressed cytokeratin, CEA, and EMA, in our experience (15 cases) and that of others.[21,32] Therefore, it would appear that immunohistochemical assessment (with the methodologic stipulations discussed above) is valuable in the differential diagnosis of carcinomas arising in the adrenal cortices, kidneys, liver, and lungs, and in their separation from amelanotic malignant melanoma (Table 4).

Because of the relatively small number of cases in this series, our results should not be considered absolutely definitive. Nevertheless, we believe that they will provide surgical pathologists with a useful adjunct to conventional microscopy in the diagnosis of adrenocortical carcinoma.

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