The Use of Immunohistochemical Expression of SF-1 and EMA in Distinguishing Adrenocortical Tumors From Renal Neoplasms

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Abstract: Steroidogenic factor -1 (SF-1) is an orphan member of the nuclear hormone receptor superfamily, and is considered to play an important role in the differentiation of steroidogenic tissues. In this study, we compared the immunohistochemical stains of SF-1 and epithelial membrane antigen (EMA) in non-neoplastic adrenal tissue, and adrenal and renal tumors using tissue microarrays (TMAs). The adrenal tissue array included 19 cases of normal adrenal cortex, 22 cases of adrenal adenoma, and 20 cases of adrenal cortical carcinoma. The renal tissue array included 20 cases of each of the following types of renal cell carcinoma: clear cell, papillary, and chromophobe. In addition, 20 cases of renal oncocytoma were also included in the study. SF-1 showed positive staining in all cases (100%) of normal adrenal cortex and adrenal cortical adenoma, and in 18 (90%) cases of adrenocortical carcinoma. In renal tumors, SF-1 showed negative stains in all of oncocytoma, papillary, and chromophobe renal cell carcinoma. Only 3 out of 20 cases of clear cell renal cell carcinoma showed weak positivity in approximately 10% of tumor cells. EMA stained positively in 85%, 95%, 100%, and 95% of clear cell, papillary, chromophobe renal cell carcinomas, and oncocytomas, respectively. EMA was completely negative in the adrenal TMAs. In conclusion, SF-1 and EMA may be helpful in the differentiation of adrenal tumors from renal tumors in difficult cases.

Key Words: steroidogenic factor-1, epithelial membrane antigen, renal, adrenal

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The adrenal mass is usually detected incidentally with 2% to 4% in general population and in 6% of abdominal computed tomography scans in patients aged 60 to 70 years old.[1,2] When a mass is detected in adrenal gland, it is important to determine whether it is benign or malignant. If it is malignant, it is critical to determine whether it is a primary adrenal lesion or metastasis.

Primary adrenocortical tumors can show a variable proportion of clear cells. An adrenal tumor with predominant clear cells is more commonly seen in an adrenocortical adenoma, whereas adrenocortical carcinoma may contain less proportion (< 25%) of clear cells.[3] Adrenocortical carcinomas are rare, aggressive tumors, with an incidence of approximately 0.72 per million individuals,[4] commonly composed primarily of cells with nonclear eosinophilic cytoplasm.[5] In addition, primary adrenal lesions can be oncocytic, no matter whether they are benign, with borderline malignant potential, or malignant.[6–8]

Renal cell carcinoma has about 25% rate of metastasis upon presentation, with the adrenal gland as one of the most frequent sites of metastasis.[9] Renal cell carcinoma can also directly involve the ipsilateral adrenal gland.[10,11] In advanced and metastatic cases, the distinction of a primary adrenal malignancy from renal tumors is important for therapeutic and prognostic reasons, but can be clinically as well as histologically challenging.[12]

An accurate pathologic diagnosis of the nature and extent of the disease is important for the planning of optimal management. For a small nonfunctional nodule of adrenal glands in a patient without a history of malignancy, it is usually sufficient to be followed by imaging; however, for a bigger mass or a patient with a history of malignancy, a definitive pathologic diagnosis may be required to make the proper treatment plan. Biopsy including fine needle aspiration, can be used as an initial procedure for such purpose.[3] However, because of the limited sampling, immunohistochemistry is often used to aid in diagnosis.

Steroidogenic factor-1 (SF-1), also known as Ad4-binding protein (Ad4BP), is an orphan member of the nuclear hormone receptor superfamily, and is considered to play an important role in the differentiation of steroidogenic tissues.[13] SF-1 is expressed in the 3 zones of the adrenal cortex, as well as in major steroidogenic tissues, such as testicular Leydig and Sertoli cells, ovarian interstitium, theca cells, and granulosa cells.[13] In adrenal cortical neoplasms, previous studies have shown SF-1 to be maintained and specifically expressed.[14] The expression of

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SF-1 has only been studied in clear cell renal cell carcinoma.[15] No study to look at the expression in other epithelial renal cell tumors has been reported. In this study, we examined SF-1 and epithelial membrane antigen (EMA) staining in both adrenal and renal epithelial tumors using tissue microarrays (TMAs).

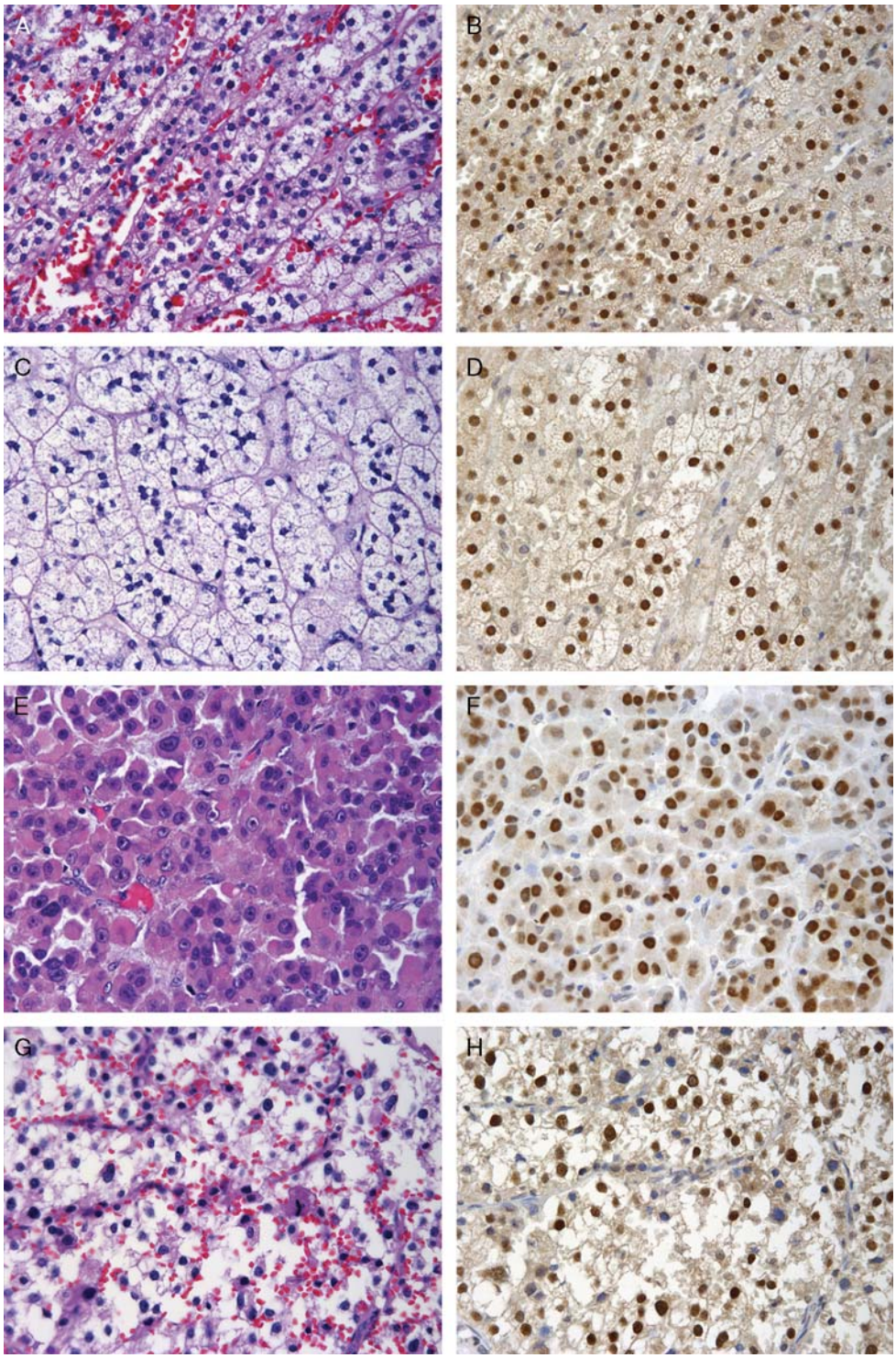

FIGURE 1. SF-1 stains in the adrenal cortical tissue and tumors, 400×. A, C, E, G: H&E stains; B, D, F, H, SF-1 immunostains. A, B: Adrenal cortical tissue; C, D: Adrenal cortical adenoma; E to H: Adrenal cortical carcinoma. H&E indicates hematoxylin and eosin; SF-1, steroidogenic factor-1.

MATERIAL AND METHODS

Renal and adrenal TMAs were constructed using formalin-fixed, paraffin-embedded tissue using a tissue arrayer (Beecher Instruments, Silver Spring, MD). Each block contains 3 representative 1.0 mm cores from the diagnostic areas of each case.

Nineteen cases of normal adrenal cortex, 22 cases of adrenal adenoma, and 20 cases of adrenocortical carcinoma from 1991 to 2009 were retrieved from the pathology archives of the Hospital of the University of Pennsylvania and used for the construction of adrenal cortical TMA.

The renal TMA represented a total of 80 renal neoplasms including 20 cases of each of the following types of tumors:Conventional (clear cell), papillary, chromophobe renal cell carcinoma, and oncocytoma.

The study was approved by the institutional review board of the University of Pennsylvania.

Immunohistochemistry was performed on the adrenal and renal TMAs. Five-micron paraffin sections of each microarray were stained with a mouse monoclonal antibody against SF-1 (dilution 1:200, clone N1665, R&D Systems, Inc., Minneapolis, MN) and EMA (dilution 1:100, clone E29, DakoCytomation, Denmark). For SF-1, antigen retrieval was achieved by incubating slides for 20 minutes at 100°C in citrate buffer. This was followed by cooling to room temperature, blocking endogenous peroxidase for 10 minutes, and incubating with Protein Block for 30 minutes at room temperature (Envision+ System, Dako, Denmark). After primary antibody incubation for 45 minutes at room temperature, slides were washed and incubated with HRP polymer for 30 minutes, washed, visualized with DAB, and counterstained with hematoxylin (Envision+ System, Dako, Denmark). No antigen retrieval was required for EMA.

Nuclear staining for SF-1 was considered positive. Intensity of staining was scored as follows: 1 = weak, 2 = moderate, 3 = strong. The percentage of positive cells was semi-quantitatively graded as focal (1, <10% of cells staining), positive (2, 10% to 70% of cells staining), and diffusely positive (3, >70% of cells staining).

For EMA, membranous staining was considered positive. Intensity and extent of staining was semi-quantitatively graded according to a similar scheme described for SF-1.

RESULTS

All tissues on the adrenal and renal TMAs were intact and available for review. SF-1 showed positive moderate-to-strong nuclear staining in all cases (100%) of normal adrenal cortex (Fig. 1A, B) and adrenal adenoma (Fig. 1C, D), and in 18/20 (90%) cases of adrenocortical carcinoma (Fig. 1E–H). The extent and intensity of staining are outlined in Table 1.

Among the renal epithelial tumors, SF-1was negative in all cases (0%) of oncocytoma and papillary and chromophobe renal cell carcinoma. Three out of 20 cases (15%) of conventional renal cell carcinomas showed weak (mean intensity 1.7) staining in approximately 10% of tumor cells.

Immunoreactivity for EMA showed frequent staining in the renal neoplasms, with positive staining in 17/20 (85%), 95% (19/20), 100% (20/20), and 95% (19/20) in the clear cell (Figs. 2A, B), papillary (Figs. 2C, D), chromophobe (Figs. 2E, F) renal cell carcinomas, and oncocytomas (Figs. 2G, H), respectively. No immunoreactivity for EMA was observed in any of the tissues in the adrenal TMA.

DISCUSSION

Nodules in the adrenal cortex are not uncommon and are frequently detected incidentally. A small nodule in a patient without history of malignancy may be simply followed clinically. Diagnostic dilemma, however, may arise for a nodule in a patient with a history of malignancy. In such cases, biopsy of the nodule may be performed to reach a pathologic diagnosis to guide the management. However, the biopsy material is usually limited and immunohistochemistry may be needed to aid in the diagnosis.

Adrenal tumors have been shown to be positive for melan A and inhibin-α; however, there is usually a significant proportion of tumors that is negative, which limit their usefulness.[13] SF-1, originally described in 1992, is a transcription factor responsible for the expression of

TABLE 1. Expression of SF-1 and EMA in Adrenal and Renal TMA

Types of Tissue	SF-1 (Positive/ total, %)	Extent (Mean)	Intensity (Mean)	EMA (Positive / Total, %)	Extent (Mean)	Intensity (Mean)
Renal TMA						
RCC						
Clear cell	3/20 (15%)	1	1	17/20 (85%)	1.7	1.65
Papillary	0/20 (0%)			19/20 (95%)	2.35	2.65
Chromophobe	0/20 (0%)			20/20 (100%)	2.8	2.9
Oncocytoma	0/20 (0%)			19/20 (95%)	2.45	2.85
Adrenal TMA						
Normal Cortex	19/19 (100%)	2.85	2.85	0/19 (0%)		
Adenoma	22/22 (100%)	2.81	2.62	0/22 (0%)		
Carcinoma	18/20 (90%)	2.4	2.35	0/20 (0%)		

RCC indicates renal cell carcinoma.

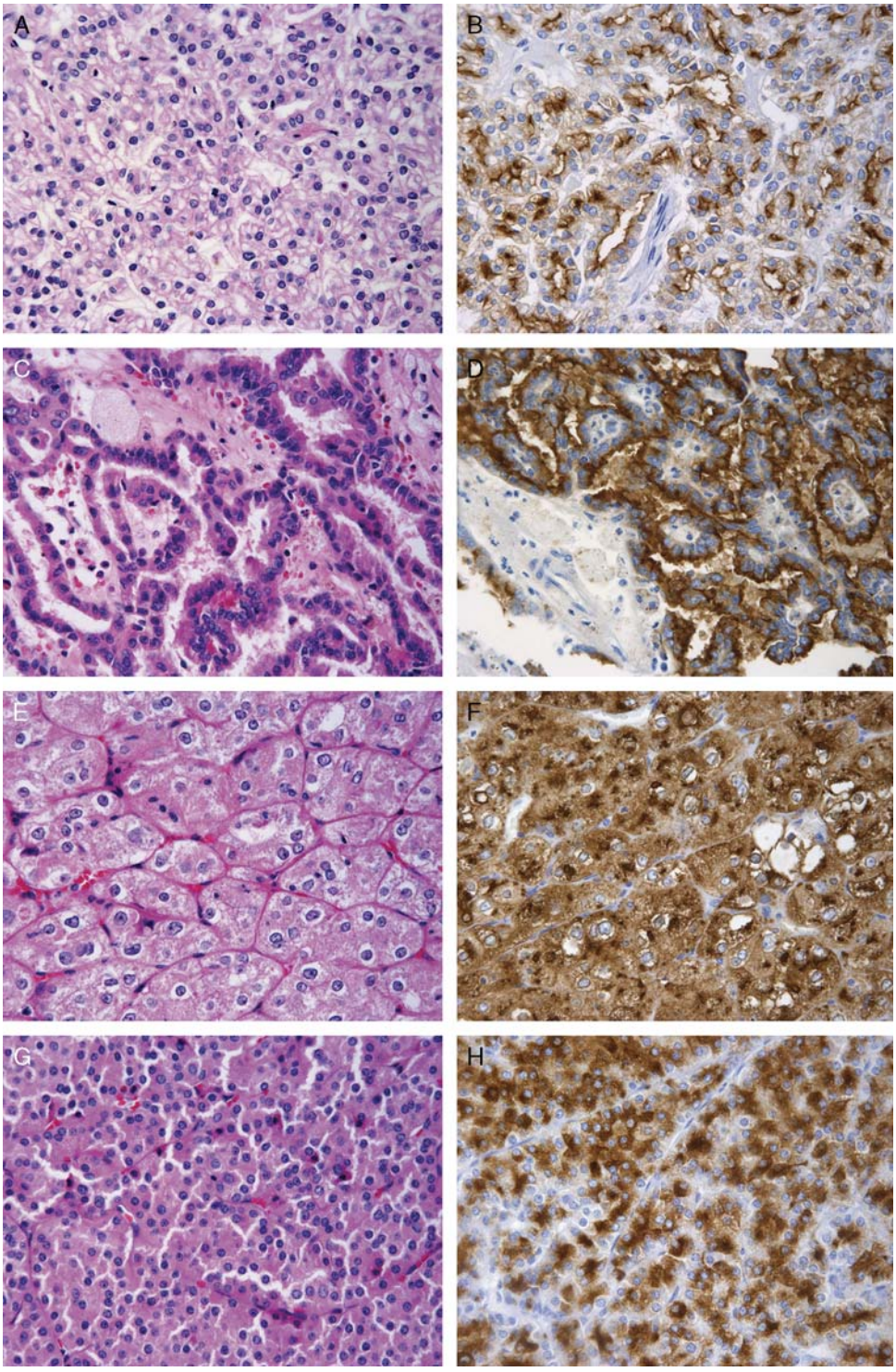

FIGURE 2. EMA stains in the renal epithelial tumors. A, C, E, G: H&E stains; B, D, F, H, EMA stains, 400×. A, B, Clear cell renal cell carcinoma; C, D: Papillary renal cell carcinoma; E, F: Chromophobe renal cell carcinoma; G, H: Oncocytoma. EMA indicates epithelial membrane antigen; H&E, hematoxylin and eosin.

the steroidogenic CYP gene encoding P450s,[16] and plays an important role in the differentiation of steroidogenic tissue.[13] In this study, we found that SF-1 showed 100% positivity in benign adrenal cortical tissue and adrenal cortical adenoma, and 90% positivity in the adrenocortical carcinoma on TMAs, proving its usefulness when tissue samples are limited, similar to previous report.[14,17–20] It is noteworthy that SF-1 also has high specificity. Sbiera

et al[14] evaluated the expression of SF-1 in 73 cases of carcinomas derived from different organs, including kidney (11 cases), lung (12 cases), breast (8 cases), colon (7 cases), pancreas (5 cases), liver (7 cases), prostate (4 cases), and ovary (3 cases) and 3 melanoma metastases, 2 lymphomas, and 1 seminoma; all of them were negative, which may serve as a marker when a metastatic tumor without known primary is encountered in pathology practice.

Adrenal cortical carcinoma is a rare but often aggressive neoplasm, with 5-year survival rates of approximately 20% and median survival of about 14 months.[21] A diagnostic dilemma in distinguishing adrenal cortical carcinomas from other tumors, especially renal cell carcinomas, can be confronted, particularly in patients with advanced disease, bulky tumors, or in cases in which the tumors are nonfunctional and do not produce the symptomatology of steroid-producing tumors. Proper diagnosis is important, as treatment may vary significantly depending on the types of tumors. From a histologic standpoint, adrenal cortical carcinomas display a wide variety of growth patterns. The tumor cells of adrenocortical carcinoma are usually nonclear and eosinophilic. Poorly differentiated tumors can exhibit marked atypia with anaplastic, bizarre, pleomorphic cells, making histogenic origin even more difficult to determine by morphology alone. The issue is further complicated by the increasing need to make the correct diagnosis on small biopsy specimens, where tissue sampling is limited. It has been proposed that clear cell composed of 25% or less as a histologic criteria to separate benign from the malignant tumor.[5,22] As such, differential diagnosis from renal cell carcinoma can be challenging. SF-1 expression was studied either only in clear cell renal cell carcinoma[15] or in unspecified type of renal cell carcinoma.[14] No immunohistochemical study has been reported in different types of renal epithelial tumors. To the best of our knowledge, this study is the first to report the SF-1 expression in different types of renal epithelial neoplasms including 20 cases each of clear cell, papillary, and chromophobe of renal cell carcinoma and oncocytoma. Our results showed that SF-1 was negative in all but only 3 cases of clear cell renal cell carcinoma. The 3 positive cases of clear cell renal cell carcinoma only showed weak nuclear positivity in approximately 10% of tumor cells.

We also examined the expression of EMA in benign adrenal cortical tissue, adrenal tumors, and renal epithelial tumors. No positivity has been detected in adrenal tissue, but 85% to 100% renal epithelial neoplasm is positive, which is similar to previous observation.[15]

In summary, we included papillary renal cell carcinoma, chromophobe renal cell carcinoma, and oncocytoma in addition to clear cell carcinoma in the study groups and compared the expression patterns of SF-1 and EMA in adrenal and renal tissue and tumors. Our results add further support to the specificity of SF-1 expression by immunohistochemistry in distinguishing adrenocortical lesions from renal epithelial tumors, particularly when used with a panel of markers including EMA. In addition, by using a TMA modality, we demonstrate the utility of SF-1 by immunohistochemistry in small samples of tissue, as often occurs with biopsies. SF-1 is a useful new marker for adrenal cortical differentiation, and shows specificity in distinguishing adrenocortical tumors from renal epithelial tumors.

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