Review

Immunohistochemical Characteristics of Adrenocortical Carcinoma: An Overview

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Received June 12, 2003; accepted July 22, 2003

Immunohistochemistry has played a major role in the practice of surgical pathology over the past three decades. These techniques have been of particular value in the diagnosis and classification of tumors of the adrenal cortex. The intermediate filament phenotype of adrenocortical carcinomas includes positivity for vimentin (80–100%), cytokeratin (40–50%) and neurofilament proteins (50%). Other markers that have proven

useful for the diagnosis of these tumors include melan A (A103), alpha inhibin, synaptophysin, calretinin, D11 and the nuclear protein adrenal 4 binding protein. The judicious use of these markers permits the distinction of adrenocortical carcinomas from other tumor types and may also be of value in the identification of the functional characteristics of these neoplasms.

Key words: neuroendocrine, melan A, cytokeratins, inhibin, differential diagnosis

I. Introduction

Adrenocortical carcinomas are rare tumors with an incidence of one to two cases per million population [8]. These tumors may be associated with glucocorticoid excess (Cushing's syndrome), sex steroid overproduction (adrenogenital syndromes), mixed syndromes of glucocorticoid and sex steroid excess or mineralocorticoid overproduction. However, a significant proportion of these tumors, approaching 75% in some series, may be unassociated with syndromes of hormone excess and typically present as large retroperitoneal mass lesions [8].

The diagnosis of non-functional adrenocortical carcinomas is often challenging, particularly in cytological samples and core biopsies [10, 28] (Fig. 1). The differential diagnosis includes pheochromocytoma, renal cell carcinoma, hepatocellular carcinoma, metastatic adenocarcinoma, liposarcoma and malignant melanoma. Immunohistochemistry has become a major approach for the diagnosis of these tumors over the past two decades [7]. The purpose of this

brief review is to provide a conceptual framework for the immunohistochemical diagnosis of adrenocortical carcinoma.

II. Intermediate Filaments

The classical immunophenotype of adrenocortical carcinoma has been reported in a number of publications as showing positivity for vimentin and either negativity or equivocal positivity for cytokeratins [5, 11, 30]. However, studies of the intermediate filament composition of these tumors have produced conflicting results. Variations in immunohistochemical results for these proteins have been ascribed to the types of tissue preparation (fresh frozen vs paraffin embedded samples), fixation protocols (alcohol based vs formalin), specificities of the antibodies, use of proteolytic enzyme digestion prior to staining and the use of heat induced antigen retrieval methods.

Wick and coworkers, for example, reported positivity for vimentin in 20 of 20 adrenocortical neoplasms (10 adenomas, 10 carcinomas) following formalin fixation with or without trypsin digestion [30] (Fig. 2). In contrast, none of the cases was positive for cytokeratins without prior trypsinization while 2 of the carcinomas and 3 of the

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Fig. 1. Adrenocortical carcinoma (hematoxylin and eosin). The tumor shows a solid to alveolar growth pattern with a moderate degree of nuclear pleomorphism.

Fig. 2. Adrenocortical carcinoma (immunoperoxidase stain for vimentin following microwave induced antigen retrieval). All of the tumor cells show immunoreactivity for vimentin.

adenomas were positive following enzymatic treatment. Miettinen *et al.* demonstrated cytokeratins in 3 of 7 (43%) adrenocortical carcinomas studied in alcohol fixed frozen or paraffin embedded sections and in 10 of 18 (55%) formalin fixed paraffin embedded sections treated with pepsin prior to staining [20]. Subsequent studies by Miettinen established that cytokeratins 8 and 18 were the most prevalent while cytokeratins 7 and 19 were present only in trace amounts [21]. Cote and coworkers, on the other hand, were unable to demonstrate cytokeratins in 13 primary or metastatic adrenocortical carcinomas (AE1, CAM5.2) fixed in formalin and subjected to protease digestion prior to staining [5].

In a study of 31 adrenocortical carcinomas fixed in formalin and treated with trypsin, Gaffey *et al.* reported that only 1 case demonstrated cytokeratin immunoreactivity

[11]. Frozen sections of this tumor and an additional previously negative tumor were positive with most cytokeratin antibodies and these results were confirmed with Western blots for cytokeratins 8 and 18. The results of this study suggested that cytokeratin expression was diminished in most adrenocortical carcinomas to levels that were too low to survive the effects of formalin fixation.

The advent of microwave induced antigen retrieval techniques, however, has shown that cytokeratins 8 and 18, as demonstrated with CAM5.2, are present in at least 40–50% of adrenocortical carcinomas, and in some instances, the staining may be extensive (DeLellis, unpublished observations; Miettinen, unpublished observations) (Fig. 3). Alternatively, the staining may be focal (Fig. 4). Occasional tumors may also be positive for broad spectrum cytokeratins, as demonstrated with AE1/AE3 (Fig. 5). Interestingly, Hoang *et al.* have reported cytokeratins in 4 of 4 formalin fixed oncocytic adrenocortical carcinomas subjected to microwave retrieval prior to staining [13].

The results of these studies underscore the fact that a significant proportion of adrenocortical carcinomas exhibit cytokeratin immunoreactivity following the use of optimized enzyme digestion or microwave induced antigen retrieval. In addition to vimentin and cytokeratin, neurofilaments are also present in these tumors. Miettinen, for example, demonstrated immunoreactivity for the 68 kD neurofilament subunit in 6 of 10 adrenocortical carcinomas [21]. The intermediate filament phenotype of these tumors, therefore, includes consistent positivity for vimentin, positivity for cytokeratins (predominantly 8 and 18) in 40–50% of cases and neurofilaments in approximately 50% of cases.

III. Neuroendocrine Markers

In addition to neurofilament subunits, adrenocortical carcinomas may contain other markers of neuroendocrine differentiation, including synaptophysin and neuron-specific enolase [12, 18, 21]. Miettinen, for example, reported synaptophysin and neuron specific enolase in 80% and 100% of cases, respectively [21] (Fig. 6). Correlative *in situ* hybridization studies have confirmed the presence of synaptophysin messenger RNA in adrenocortical carcinomas [18]. Stains for chromogranin, on the other hand, are negative in adrenocortical carcinomas while pheochromocytomas typically demonstrate a synaptophysin and chromogranin positive phenotype.

IV. Melan A

Melan A (MART-1) is a melanocyte differentiation antigen recognized by autologous T-lymphocytes [6, 7]. This antigen can be demonstrated in formalin fixed specimens by the monoclonal antibody A103 which was produced against melan A recombinant protein. The studies of Chen *et al.* and Jungbluth and coworkers established the value of this antibody as a sensitive and specific marker for normal and neoplastic melanocytic cells [4, 16]. Subsequent studies

Fig. 3. Adrenocortical carcinoma (immunoperoxidase stain for cytokeratins 8 and 18 using the monoclonal antibody CAM 5.2 following microwave induced antigen retrieval). All of the tumor cells show positive staining with accentuation in the perinuclear region.

Fig. 4. Adrenocortical carcinoma (immunoperoxidase stain for cytokeratins 8 and 18 using the monoclonal antibody CAM 5.2 following microwave induced antigen retrieval). The tumor shows only focal dot-like perinuclear immunoreactivity.

Fig. 5. Adrenocortical carcinoma (immunoperoxidase stain for broad spectrum cytokeratins using a combination of the monoclonal antibodies AE1 and AE3 following microwave induced antigen retrieval). Most of the tumor cells in this field are positive.

Fig. 6. Adrenocortical carcinoma (immunoperoxidase stain for synaptophysin following microwave induced antigen retrieval). All of the tumor cells show positive staining.

demonstrated that A103 immunoreactivity was also present in a limited number of other cell types including adrenocortical cells, testicular and ovarian Leydig cells, Sertoli cells, theca and granulosa cells of the ovarian follicles and luteal cells [3]. Busam and coworkers demonstrated that A103 immunoreactivity was also present in 100% (29/29) of adrenocortical carcinomas and 100% (5/5) adrenocortical adenomas while pheochromocytomas, carcinomas of diverse origins and germ cell tumors were negative [3] (Fig. 7). Renshaw and Granter confirmed the utility of A103 as a marker for adrenocortical tumors but reported a lower sensitivity than that reported by Busam *et al.* [24]. Since that time, A103 has been reported only rarely in non-melanocytic

and non-steroid producing tumors [14]. The results of these various studies have confirmed the value of A103 as a particularly useful marker for adrenocortical carcinomas [28].

Studies utilizing reverse transcriptase PCR to demonstrate melan A mRNA in adrenal cortical tissue, however, have been unsuccessful. This observation has led to the conclusion that the immunoreactivity of A103 in cortical cells is due to a cross reaction with an epitope different from that present in melanoma cells. This conclusion is supported by the fact that other antibodies to melan A fail to react with steroid producing cells in the adrenal cortex and other sites [4, 6, 28].

Fig. 7. Adrenocortical carcinoma (immunoperoxidase stain for melan A using the monoclonal antibody A103 following microwave induced antigen retrieval). All of the tumor cells show strong staining.

Fig. 8. Adrenocortical carcinoma (immunoperoxidase stain for inhibin A following microwave induced antigen retrieval). Some of the cells are strongly stained while others are weakly stained or negative.

V. Inhibins

The inhibins are glycoprotein hormones which are present in a variety of steroid producing cells and which inhibit the secretion of follicle stimulating hormone. Immunohistochemical studies have established the utility of alpha inhibin as a sensitive and specific marker for adrenocortical carcinomas [1, 10, 23] (Fig. 8). In the study reported by Pelkey *et al.*, alpha inhibin immunoreactivity was present in 12 of 15 (80%) of adrenocortical carcinomas [23]. Immunoreactivity for this marker was strong in 78% of tumors associated with virilization or Cushing's syndrome while only 14% of tumors that were non-functional or associated with hyperaldosteronism were strongly reactive. Similar results have also been reported by Arola *et al.* [1, 2].

Munro and coworkers reported positivity for alpha inhibin and beta A and B subunits in most adrenocortical tumors [22]. There were no significant differences in alpha inhibin expression between benign and malignant adrenocortical tumors although Munro *et al.* suggested that loss of alpha inhibin in a subgroup of carcinomas might indicate a role in tumor progression. Renshaw and Granter have compared the sensitivities and specificities of alpha inhibin and melan A (A103) and have concluded that A103 is slightly more specific and alpha inhibin slightly more sensitive for the diagnosis of adrenocortical tumors [24].

VI. Calretinin

Calretinin is a calcium binding protein which has been used most extensively as a marker for mesotheliomas [9]. The studies of Doglioni *et al.* noted that this protein was present in certain steroid producing neoplasms, including ovarian stromal tumors [9]. Based on this observation, Jorda *et al.* have studied the distribution of calretinin in adrenal

cortical neoplasms [15]. In their series of adrenocortical carcinomas, immunoreactive calretinin was present in 8 of 12 adrenocortical carcinomas and 16 of 21 adenomas. The sensitivity and specificity of this marker were 73% and 100%, respectively. A correlative analysis of inhibin in the same group of tumors yielded sensitivities and specificities of 70% and 100%, respectively, while the combination of calretinin and inhibin yielded sensitivities and specificities of 94% and 100%, respectively.

VII. Monoclonal Antibody D11

The monoclonal antibody D11, was introduced as a marker for adrenal cortical neoplasms by Schroder *et al.* [27]. The antibody recognizes several 59 kD proteins capable of binding apolipoprotein E. Schroder *et al.* demonstrated nuclear positivity in 100% of benign (57 cases) and malignant (15 cases) adrenocortical tumors [27]. A subsequent study by Tartour *et al.* demonstrated nuclear positivity in 44% (8 of 18 cases) of carcinomas, all of which were well differentiated [29]. The staining pattern was focal (less than 20%) in 2 of 8 cases and diffuse in the remaining 6 cases. Nuclear staining was accompanied by varying degrees of cytoplasmic staining. Four additional cases showed cytoplasmic staining without nuclear staining while 6 cases were entirely negative. Cytoplasmic staining for D11, however, is not specific for adrenocortical carcinomas since it was also present in 5/18 (27.7%) of metastases to the adrenals, 20/20 (100%) of hepatocellular carcinomas, 3/5 (60%) of lung carcinomas and 1/50 (2%) of renal cell carcinomas [29]. Nuclear reactivity for D11, therefore, has a high specificity but relatively low sensitivity for the diagnosis of adrenocortical carcinomas. In a more recent study, Komminoth *et al.* reported D11 immunoreactivity in 22/27 (81%) of adrenocortical carcinomas [18].

Table 1. *Differential diagnosis of adrenal cortical carcinoma*

	CK	VIM	EMA	CEA	S100	HBM45	MEL	NF	SYN	CGA	AFP	CAL	INH	RCC	HEPPAR-1
Adrenal Cortical Ca	+/-	+	-	-	-/+	-	+	+	+	-	-	+	+	-	-
Pheochromocytoma	-	+/-	-	-	+	-/+	-	+	+	+	-	-	-	-	-
Renal Cell Ca	+	+	+	-	+/-	-	-	-	-	-	-	-/+	-/+	+	-
Hepatocellular Ca	+	+	+	+	+/-	-	-	-	-	-	+	-/+	-/+	-	+
Metastatic Adeno Ca	+	+/-	+	+	+/-	-	-	-	-	-	-	-/+	-	-	-
Liposarcoma	-	+	-	-	+	-	-/+	-	-	-	-	-	-	-	-
Malignant melanoma	-/+	+	-	-	+	+	+	-	-	-	-	-	-	-	-

Key: +, positive; +/-, predominantly positive; -/+, predominantly negative; -, negative.

CK, cytokeratin (CAM 5.2); VIM, vimentin; EMA, epithelial membrane antigen; MEL, melan A (A103); NF, neurofilament; SYN, synaptophysin; CGA, chromogranin A; AFP, alpha fetoprotein; CAL, calretinin; INH, inhibin A; RCC, renal cell carcinoma antigen; HEPPAR-1, hepatocyte specific antibody.

VIII. Transcription Factors and Steroidogenic Enzymes

The nuclear protein adrenal 4 binding protein (Ad4BP) or steroid factor 1 (SF-1) is a transcription factor that has also been used for the diagnosis of adrenocortical carcinomas [26]. This marker has been demonstrated in 8/8 (100%) of adrenocortical carcinomas but is absent from hepatocellular carcinomas, melanomas, ovarian and uterine clear cell carcinomas, large cell bronchogenic carcinomas and pheochromocytomas. Ad4BP has also been reported in Leydig cells of the testis, thecal and granulosa cells of the ovary and gonadotropin producing cells of the adenohypophysis.

Antibodies to steroidogenic hormones have also been used in immunohistochemical formats for the study of adrenocortical carcinomas. Sasano *et al.*, for example, have demonstrated that a number of cells in adrenocortical carcinomas did not express all of the enzymes required for the synthesis of biologically active steroids [25]. This observation may, in fact, be responsible for the fact that increased levels of precursor steroid are present in adrenocortical carcinomas. However, there have been relatively few applications of immunohistochemical methods for steroidogenic enzymes in the diagnostic setting.

IX. Other Markers

A variety of other markers have been employed to differentiate adrenocortical carcinomas from other tumor types (Table 1). For example, most renal cell carcinomas express Lewis blood group isoantigens (e.g. Lewis X) while adrenocortical carcinomas do not [5]. Most adenocarcinomas are positive for carcinoembryonic antigen, epithelial membrane antigen and CD15 while adrenocortical carcinomas are negative [7, 27]. Adrenocortical carcinomas are also negative with the renal cell carcinoma (RCC) and hepatocyte antibodies [19, 31] (Table 1).

X. References

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