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Original Research

Cytokeratin 7 and antimitochondrial antibody expressions in the differential diagnosis of renal epithelial neoplasms with eosinophilic cytoplasm

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Abstract

Objectives: The distinction between epithelial renal tumors with eosinophilic cytoplasm, namely, chromophobe renal cell carcinoma (ChRCC), clear cell (conventional) renal cell carcinoma (ECRCC) with eosinophilic cytoplasm, and oncocytoma may remain questionable in some cases because of overlapping morphologic features. We evaluated cytokeratin 7 (CK 7) and antimitochondrial antibody (AMA) expressions to determine their potential value in distinguishing these tumors.

Methods: In this study, 24 renal tumors were included. They consisted of 9 ChRCCs, 11 oncocytomas and 4 ECRCCs. Immunoperoxidase staining for CK 7 and AMA were performed.

Results: *CK 7 staining:* All ChRCCs showed strong cytoplasmic immunoreactivity with conspicuous peripheral accentuation. All ECRCCs and oncocytomas were negative for CK 7.

AMA staining: All ChRCCs showed diffuse cytoplasmic, coarse-granular immunostaining with peripheral accentuation. In all ECRCCs, diffuse cytoplasmic was observed with randomly distributed coarse-granular staining. All oncocytomas demonstrated diffuse cytoplasmic, but fine-granular staining.

Combined interpretation of CK 7 and AMA stainings: Seven of eight ChRCCs showed combined AMA (+) with diffuse cytoplasmic coarse-granular with peripheral accentuation and CK 7 (+). All oncocytomas were CK 7 (-) and AMA (+). All ECRCCs was also CK 7 (-) and AMA (+) like oncocytomas, but the staining pattern of AMA was randomly distributed, and coarse-granular, rather than fine-granular which was observed in all of the oncocytomas.

Conclusions: These results suggest that the combined interpretation of CK 7 and AMA may provide a better approach in the differential diagnosis of renal epithelial tumors with eosinophilic cytoplasm, in addition to the relatively well-known distinctive immunostaining patterns of AMA.

INTRODUCTION

Renal epithelial tumors with predominantly or exclusively eosinophilic cytoplasm include chromophobe renal cell carcinoma (ChRCC), clear cell (conventional) renal cell carcinoma (ECRCC) with eosinophilic cytoplasm, type 2 papillary renal cell carcinoma and oncocytoma. The most challenging differential diagnosis to be made among them includes oncocytoma, ChRCC and ECRCC. Renal oncocytoma is a benign neoplasm, while ChRCC and ECRCC are malignant tumors. ChRCC and ECRCC have some overlapping histological features with renal oncocytoma, such as cellular pleomorphism and perirenal adipose tissue invasion as well as vascular invasion. The overall prognosis and biological behavior of CRCC are significantly better than ECRCC [1, 2]. Therefore, making the distinction among these eosinophilic tumors is crucial. These tumors may not be classified with certainty using conventional light

microscopy alone even in the hands of experienced pathologists.

Many immunohistochemical markers, such as cytokeratin (CK) 7, CK14 and CK20, epithelial membrane antigen (EMA), CD10, renal cell carcinoma antigen, E cadherin, kidney spesific cadherin (KSC), parvalbumin, caveolin 1,CD63 and GLUT1 as well as Hale's colloidal iron as a histochemical method have been reported to be useful and contributory in distinguishing of these tumors [1-8, 12]. However, their sensitivity and specifity are varying degrees in diagnosing renal epithelial tumors with eosinophilic cytoplasm. Ultrastructural demonstration of intracytoplasmic microvesicles and mitochondria for the diagnosis of ChRCCs and oncocytomas, respectively, is still thought to be the most reliable method [2].

Our aim was to evaluate potential value in immunostaining patterns using the cytokeratin 7 (CK 7) and antimitochondrial antibody (AMA) and to assess their potential role in the differential diagnosis of most challenging eosinophilic renal epithelial tumors.

MATERIALS AND METHODS

A total of 24 renal epithelial tumors with eosinophilic cytoplasms consisting of 12 ChRCCs, 9 oncocytomas and 3 ECRCCs were firstly included in this study. When we reviewed reassessed H&E sections of all cases retrospectively and collectively under the guidance of imunohistochemistry, we concluded that three of the ChRCCs should have been diagnosed as oncocytomas and two of them, in return, must have been the ECRCCs. Similarly, one ECRCC should actually be ChRCC. Lastly, only one of the nine oncocytomas showed an immunohistochemical labeling pattern consistent with the ChRCC. Hence, we need an amendment in the former diagnoses, and re-grouped the cases according to the interpretation based on the CK 7 and AMA immunoreactivities. As a result of, a total of 24 renal epithelial tumors consisted of 9 ChRCCs, 11 oncocytomas and 4 ECRCCs, according to this amended list.

Immunohistochemical study was performed on 10% buffered formalin-fixed, paraffin-embedded tumor tissue samples of these cases. Consecutive sections from the most representative paraffin blocks of each case were submitted to study for cytokeratin 7 (CK 7) and antimithochondrial antibody (AMA), immunohistochemically. Labvision automated immunostainer (Labvision Corporation, Fremont, CA, USA) utilizing standard streptavidin-biotin method was used for the procedure. Briefly**,** after deparaffinization, hydration, endogenous peroxidase blocking, and heat-induced antigen retrieval steps; the tissue sections were incubated for 30 minutes at room temperature with the CK 7 marker (Neomarkers, Fremont, CA, USA; dilution 1:80) and AMA (Ab2 clone OV-TL 12/30;Neomarkers, Fremont, CA, USA; dilution 1:100). The AMA monoclonal antibody used in this study, Ab2 (clone MTCO2), recognizes a 60-kDa nonglycosylated protein component of mitochondria found in human cells. Non-neoplastic kidney parenchyma adjacent to the tumors was used as positive internal controls. Neoplastic or non-neoplastic stromal cells were used as negative internal controls.

For each case, the semi-quantitatively estimated percentage of stained tumor cells and the staining intensity for two markers were evaluated and recorded by all of the authors independently. Intensity was classified as follows: 0 = no stain; 1+ = weak; 2+ = moderate; and 3 + = Strong. A positive result was defined as strong staining of more than 5% of tumor cells.[8] Immunohistochemical assessments were carried out with the correlation of corresponding H&E sections.

RESULTS

Immunohistochemical Profiles in Renal Epithelial Neoplasm's with Eosinophilic Cytoplasm:

The quality of immunostainings was excellent and the expression of each marker in routinely processed consecutive tissue sections was observed on a clean background. The immunoreactivity showed distinctive cytoplasmic distribution patterns for both CK 7 and AMA with a membranous accentuation.

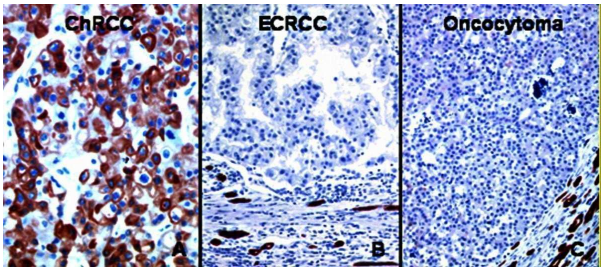

Figure 1 A-C. CK7 expression patterns among the renal epithelial tumors with eosinophilic cytoplasm

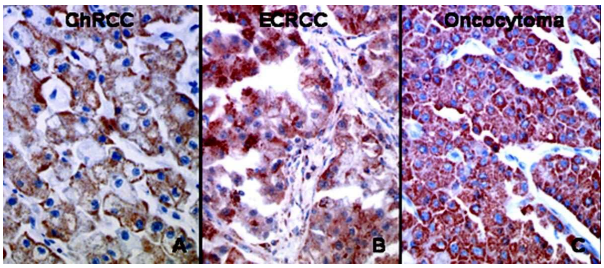

Figure 2 A-C. AMA expression patterns among the renal epithelial tumors with eosinophilic cytoplasm.

Table 1. CK 7 and AMA expressions among the renal epithelial tumors with eosinophilic cytoplasm.

Diagnoses	CK 7 N (%)	AMA N (%) Staining patterns		
		Diffuse coarse-granular with peripheral accentuation	Diffuse randomly distributed coarse-granular	Diffuse fine-granular
ChRCC (N=9)	9 (100)	9 (100)		
ECRCC (N=3)	0 (0)		3 (100)	
Oncocytoma (N=11)	0*(0)	-		11(100)

ChRCC: Chromophobe renal cell carcinoma, **ECRCC:** Clear cell renal cell carcinoma with eosinophilic cytoplasm

*: Seven cases from the oncocytoma group were dismissed due to the scarce (less than 5%) staining of the tumor cell population.

Table 2. The combined CK 7 and AMA expressions among the renal epithelial tumors with eosinophilic cytoplasm.

CK7	AMA			ChRCC (N=9)	ECRCC (N=3)	Oncocytoma (N=11)
	Diffuse coarse-granular with peripheral accentuation	Diffuse randomly distributed coarse-granular	Diffuse fine-granular			
+	+			7	-	-
+		+		2	-	-
+			+	-	-	-
-	+			-	-	-
-		+		-	3	-
-			+	-	-	11

ChRCC: Chromophobe renal cell carcinoma, **ECRCC:** Clear cell renal cell carcinoma with eosinophilic cytoplasm

Cytokeratin 7 (CK 7):

Ten to ninety-five percent of the tumor cells showed strong cytoplasmic staining with peripheral cell membrane accentuation in all cases. In non-neoplastic distal renal tubules and collecting ducts, diffuse fine-granular cytoplasmic immunoreactivity was accepted as positive internal control. All ChRCCs showed strong cytoplasmic immunoreactivity with conspicuous peripheral accentuation (Table 1) (Figure 1A). All ECRCCs was negative for CK 7 (Figure 1B). Among the eleven oncocytomas, four cases were completely negative (Figure 1C) and seven cases showed only focal staining in less than 5% of the tumor.

Anti-mithochondrial antibody (AMA):

All tumors were positive for AMA, but showed distinctive cytoplasmic immunostaining patterns among the groups (Table 1). All ChRCCs showed diffuse cytoplasmic coarse-granular immunostaining with peripheral accentuation (Figure 2A). All ECRCCs was positive with diffuse cytoplasmic but randomly distributed coarse-granular immunoreactivity (Figure 2B) and in all oncocytomas, diffuse cytoplasmic, but fine-granular staining was observed (Figure 2C).

Combined interpretation of CK 7 and AMA:

Seven of eight ChRCCs showed both CK 7 (+) and AMA (+) expressions (Table 2). AMA staining in these cases was diffuse cytoplasmic coarse-granular pattern with peripheral accentuation. All oncocytomas were CK 7 (-) and AMA (+). All ECRCCs was also CK 7 (-) and AMA (+) like oncocytomas, but the staining pattern of AMA was randomly distributed coarse-granular, rather than fine-granular which was observed in oncocytomas.

DISCUSSION

Most renal epithelial tumors can be diagnosed reliably by the experts on the basis of morphology alone. However, there are morphological overlaps among the eosinophilic subtypes of the renal cell carcinomas that can be problematic even for very well experienced pathologists. The most challenging cases are usually ChRCC, oncocytomas, and the ECRCC. While renal oncocytoma is accepted as a benign tumor, ChRCC has low malignant potential and ECRCC usually shows an aggressive clinical course. The exact discrimination of subtypes is crucial because of their different biologic

behaviors. Some ancillary methods, which have their pros and cons, are being used for this purpose. For instance, chromophobe RCC can be recognized by identifying its peculiar microvesicular structures using electron microscopy, but this opportunity may not always be available in most centers, and routine tissue processing procedures may cause decomposition of these peculiar structures [9]. On the other hand, Hale's colloidal iron stain is positive in ChRCC and is negative or only focally positive in others [2]. However, this histochemical staining has some technical drawbacks, which prevent its widespread usage. Therefore, methods that would be done without any prerequisites and be adopted by all of the surgical pathology laboratories are needed. For this reason, we propose the immunohistochemical investigation of CK7 and AMA, and their co-interpretation in the differential diagnosis of renal epithelial tumors with eosinophilic cytoplasm.

Antimitochondrial antibody was previously used to demonstrate the presence of mitochondria in normal tissues and in tumors such as renal oncocytoma and RCC [1, 10, 11]. The distinctive immunoreactivity patterns of antimitochondrial antibody (clone 113-1) in the differential diagnosis of renal epithelial tumors with eosinophilic cytoplasm have been first noticed by Tickoo et al [1] In their study, the distribution pattern of AMA was characteristically found as diffuse and coarse-granular with an evident peripheral accentuation in all of the ChRCCs (100%) while the distribution and quality of staining was diffuse fine granular in renal oncocytoma (96%) and diffuse but randomly scattered coarse-granular in ECRCCs (100%). Even though the peripheral condensation was occasionally seen in renal oncocytoma and ECRCC, distinction among these tumors was still thought to be possible because of their fairly different properties of immunoreactivity. Mete et al observed similar patterns in their study as diffuse coarse-granular staining with peripheral accentuation in 96% of ChRCCs, diffuse randomly distributed coarse-granular staining in 82% of ECRCC and diffuse fine-granular staining in 91% of oncocytomas [5]. Our observations considering the quality and distribution patterns of immunoreactivity were concordant with the previously mentioned studies (Table 1).

Leroy et al observed diffuse strong cytoplasmic CK 7 expression with peripheral membranous accentuation in proportion ranged from 90 to 100% of tumor cells of all ChRCC, while weaker and focal cytoplasmic CK 7 expression in less than 5% of oncocytic cells without membranous highlighting in only 28% of oncocytomas. Remaining oncocytomas (72%) showed no immunoreactivity for CK 7 [12]. Abrahams et al observed diffuse strong cytoplasmic CK 7 expression with membranous augmentation in proportion ranged from 50 to 80% of tumor cells in about 83% of ChRCC, while focal cytoplasmic CK 7 expression in 50% of oncocytomas, and 100% of the other RCCs [13]. Mathers et al observed patchy and weak to moderate cytoplasmic expression of CK 7 in all oncocytomas in contrast to the completely negative immunoreactivity in all ChRCCs [8]. Gerharz et al reported that all of the ChRCCs were positive, but oncocytomas did not show any immunoreactivity with CK 7, in return [14].

In the present study, we clearly observed the importance of staining patterns of CK 7 and AMA immunoreactivity among renal epithelial tumors with eosinophilic cytoplasm. This observation is concordant with the findings reported in previous studies [1-10]. We suggest that one must take into consideration the following points in interpreting the CK 7 and AMA immunostainings: 1) The threshold for the extent of staining to be accepted as significant, which is commonly assumed as greater than 5% 2) The relative proportion or percentage of stained cells, i.e. focal, extensive, and diffuse 3) The intensity of staining, such as faint, weak, or strong 4) Presence or absence the peripheral cytoplasmic accentuation, and 5) The overall properties of immunoreactivity, i.e. fine-granular, coarse-granular, evenly or randomly distributed. An important point that must always be kept in mind is not to forget the normal structures entrapped in the tumor when interpreting the immunohistochemisty. Therefore, whenever a doubtful or unexpected staining is encountered, a correlation with standard H&E sections should be made.

In conclusion, using CK 7 and AMA together, rather than separately, may provide more reliable discrimination in the differential diagnoses of renal epithelial tumors with eosinophilic cytoplasm. Diffuse positivity of CK 7 with membranous highlighting and AMA with diffuse, coarse-granular staining with peripheral accentuation strongly favors ChRCC. Among the remaining cases showing no or focal immunoactivity for CK 7, diffuse randomly distributed AMA positivity supports the ECRCC, while diffuse fine-granular staining of AMA favors oncocytoma.

The authors declare that they have no conflict of interest.

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