Original Research

Expression of PAX2 and Renal Cell Carcinoma Antigen in Mucoepidermoid Carcinoma

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Clear cell renal cell carcinoma (CCRCC) is the most common metastatic clear cell tumor in the head and neck. The most common primary tumor of the head and neck with clear cell morphology is mucoepidermoid carcinoma (MEC). The distinction between MEC with clear cells (CMEC) and metastatic CCRCC can be challenging in a small biopsy specimen. Expression of PAX2 and renal cell carcinoma antigen (RCCma) has been widely used to aid of diagnosis for both primary and metastatic RCC. The aim of this study is to evaluate the utility of expression of PAX2 and RCCma between CMEC and metastatic CCRCC in a clinical setting using tissue microarrays (TMAs). In primary CCRCC, the nuclear immunoreactivity for PAX2 was found in 47 of 120 cases (39%), and the membranous staining pattern for RCCma was revealed in 69 of 120 cases (58%). The immunostain profiles of metastatic RCC showed positive staining for PAX2 in 21 of 94 cases (22%) and RCCma in 19 cases (20%), respectively. Two of six cases (33%) of metastatic RCC to the head and neck region display immunoreactivity for either PAX2 or RCCma. For MEC, positive membranous and cytoplasmic staining of RCCma was found in 3 of 23 cases (13%), and diffuse cytoplasmic reactivity for PAX2 was noted in 19 cases (83%). However, none of MEC showed nuclear reactivity that is specific for PAX2. Results of our study suggest that although PAX2 and RCCma are relatively specific for CCRCC, one should be cautious when interpreting the results of RCCma and PAX2 expression in the setting of CMEC versus metastatic CCRCC, particularly in a biopsy specimen. Clinicopathologic correlation combined with histomorphology and a panel of immunohistochemical markers is essential to render correct diagnosis.
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Key Words: *Mucoepidermoid carcinoma, clear cell renal cell carcinoma, PAX2, RCCma, differential diagnoses*

INTRODUCTION

Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of salivary origin. It represents about 20 and 34 percent of malignant tumors in the major and minor salivary gland, respectively.[1-3] MEC is a heterogeneous neoplasm composed of variable proportions of mucinous, epidermoid, intermediate, columnar, and clear cells organized in solid and cystic growth patterns.[4,5] In most of MEC, clear cells account for about 10 percent of tumor cells, but can comprise a large portion of the tumor in some rare cases.[5] The distinction of MEC with predominant clear cell morphology (CMEC) from metastatic clear cell renal cell carcinoma (CCRCC) can be a diagnostic challenge in some cases, particularly with a small biopsy specimen.

Expression of renal cell carcinoma antigen (RCCma) has been widely used as an aid in the diagnosis of both primary and metastatic RCC.[6-8] In addition, expression of PAX2, a transcriptional factor of paired-box family expressed during the development of epithelial and mesenchymal components in urogenital system, has recently been shown to have a higher specificity and sensitivity for metastatic RCC than RCCma.[9-10] The role of these two markers in the differential diagnosis of CMEC and metastatic CCRCC, however, has not been explored. The aim of this study is to evaluate the utility of the expression of RCCma and PAX2 for the most common metastatic clear cell tumor in the head and neck, CCRCC, versus MEC, the most common primary clear cell tumor in the head and neck region.

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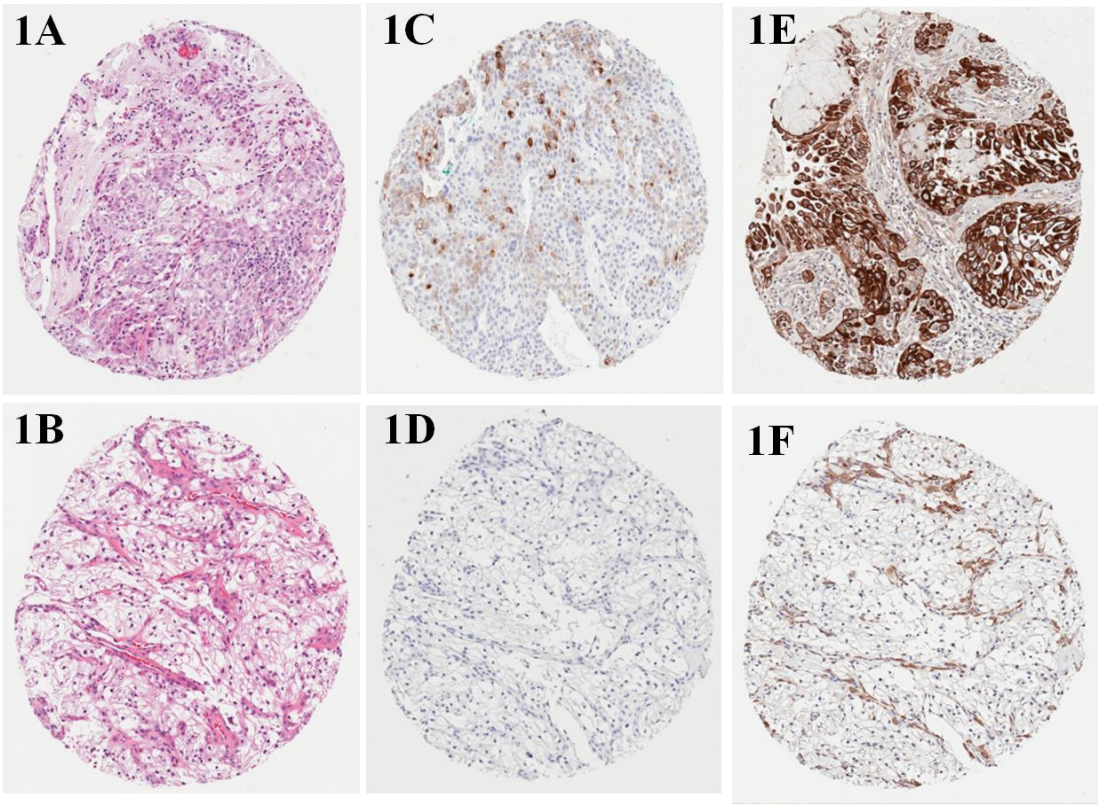

Figure 1. Immunostaining pattern of RCCma and PAX2 in mucoepidermoid carcinoma (MEC). **1A** and **1B:** H & E sections of MEC containing predominantly epidermoid component (**1A**) and clear cell component (**1B**); The immunoreactivity for RCCma is detected on both cytoplasm and membrane of the tumor cells. The positive staining is predominantly on the epidermoid component (**1C**) and less in the clear cell component (**1D**). The cytoplasmic staining of PAX2 was found in both epidermoid (**1E**) and clear cell (**1F**) components of MEC with former showing strong and diffuse reactivity.

METHODS

Patients

All patients for this study were diagnosed with either MEC or primary or metastatic RCC at Roswell Park Cancer Institute (RPCI) from 2000 to 2010. A total of twenty-three patients with MEC were identified, 11 of major salivary gland origin (parotid and submaxillary glands), 11 of minor salivary gland origin, and 1 involving a cervical lymph node. Tumors were graded according to the criteria by World Health Organization as low, intermediate and high-grade lesions. In addition, one hundred twenty patients with primary clear cell RCC and ninety-four patients with metastatic RCC were identified for the study. This study was approved by Institution Review Board at RPCI.

Tissue Microarray and Immunohistochemistry

Tissue blocks from identified cases of MEC and RCC were used to construct tissue microarray (TMA) as previously described.[11] Three tissue cores, 0.6 mm in diameter, were taken from a representative area of each case and used to assemble the arrays. Five-micrometer sections were cut from the TMA and subjected to immunohistochemical studies. Quality of histomorphology of the TMA was assessed on H & E stained sections before immunohistochemical staining. Paraffin sections were cut at 5μm, placed on charged slides, and dried at 60℃ for one hour. Slides were cooled to room temperature, deparaffinized in three changes of xylene, and rehydrated using graded alcohols. For antigen retrieval, slides were heated in the microwave for 20 minutes in citrate buffer, followed by a 15 minute cool down and a PBS/T wash. Endogenous peroxidase was quenched with aqueous 3% H_2O_2 for ten minutes and washed with PBS/T. Slides were then loaded on the Dako Autostainer and blocked for five minutes with a serum-free protein block (Dako). The primary mouse monoclonal antibody PAX2 (Lifespan Biosciences) was applied at a concentration of 1 μg/ml for one hour. An isotype-matched control (1 μg/ml mouse IgG2a) was used on a duplicate slide in place of the primary antibody as a negative control. A PBS/T wash was followed by incubation with biotinylated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc.) for 30 minutes, another PBS/T wash and application of the Elite ABC Kit (Vectastain). Slides were washed with PBS/T and the DAB chromagen (Dako) was applied for 5 minutes. The same general procedures were used for the mouse monoclonal antibody RCCma (Thermo Scientific, Fremont, CA) with the following exceptions: antigen retrieval consisted of target

retrieval solution (Dako) in a vegetable steamer for 40 minutes followed by a 20 minute cool down. Mouse IgG1 (1 µg/ml) was used as an isotype control, and subsequently anti-mouse Envision+ reagent (Dako) and DAB+ chromagen (Dako) were applied. After immunohistochemical staining, slides were counterstained with Hematoxylin, dehydrated, cleared and coverslipped.

Scoring of Immunostains

TMA sections with satisfactory immunostains for RCCma and PAX2 were scanned by Spectrum automated microscope (Aperio Technologies, Vista, CA). Sections were scored using semi-quantitative scale for each individual tissue cores on the TMA slides. Sub-cellular (membrane, cytoplasm or nuclear) expression of RCCma and PAX2 was evaluated by two independent pathologists (CM and BX). Immunostain intensity (ranging from 0 to 3 with 0 = negative, 1 = weak, 2 = moderate, 3 = strong) and percentage of positive tumor cells (1 < 10%, 2 = 11-30%, 3 > 30%) were recorded. The final score was the product of multiplying the staining intensity by percentage of positive cells and expressed as + for score 1-3; ++ for score 4-6 and +++ for score 7-9.

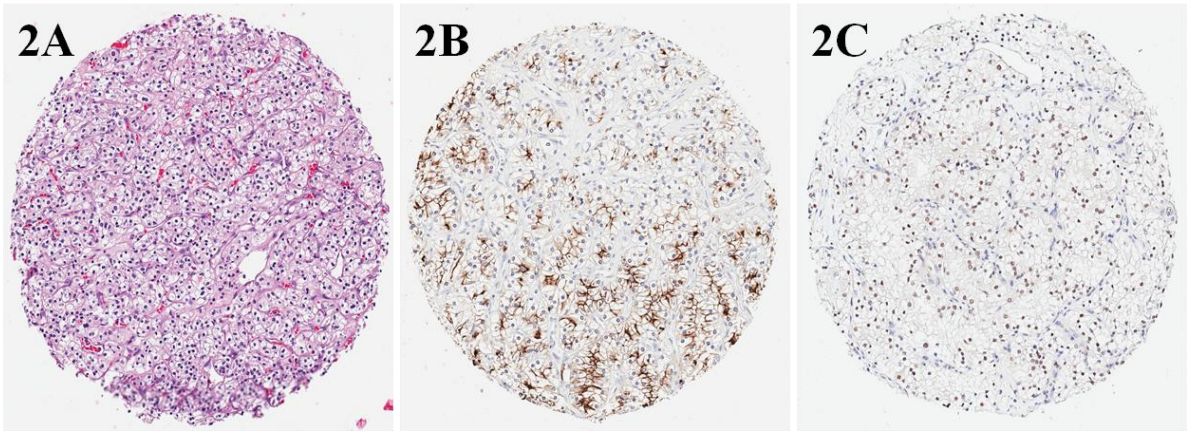

Figure 2. Expression of RCCma and PAX2 in primary renal cell carcinoma (RCC). **2A:** H & E section of RCC; **2B:** Strong membranous staining pattern for RCCma; **2C:** Specific nuclear staining for PAX2.

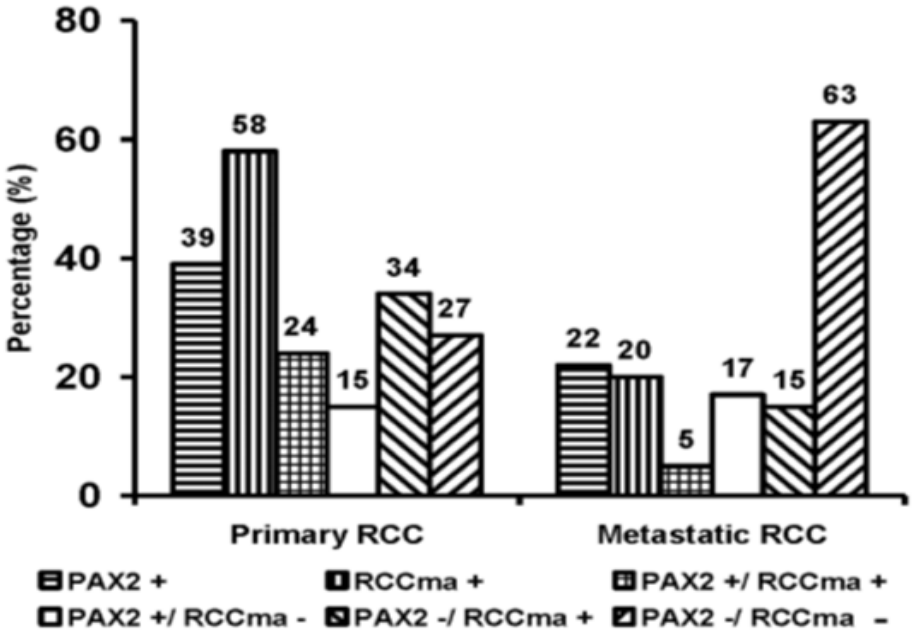

Figure 3. Immunoreactivity of PAX2 and RCCma in primary and metastatic RCC TMAs.

RESULTS

The 23 cases of MEC were classified according to their histological grade of malignancy in low grade (14 cases, 61%), intermediate grade (5 case, 22%) and high grade (4 case, 17%). Eleven cases (48%) show variable clear cell component (CMEC), ranging from 20-60% of the tumor. Among the CMEC, 9 cases were low grade (82%), 1 intermediate (9%) and 1 high grade (9%). Immunohistochemical staining results show that RCCma was positive in three of the eleven CMEC (27%) with variable degree of membranous and cytoplasmic staining. All RCCma positive cases were low grade and staining is primarily involving the epidermoid cell population (**Table 1**, **Figure 1C-1D**). Immunohistochemical studies for PAX2 revealed 19 cases (83%) with diffuse cytoplasmic reactivity and higher intensity in the epidermoid cells (**Figure 1E-1F**, **Table 1**). However, no cases showed nuclear staining that is specific for PAX2.

For primary RCC, the membranous staining pattern for RCCma was demonstrated in 69 of 120 cases (58%) (**Figure 2B** and **Figure 3**). PAX2 nuclear staining was found in 47 of 120 cases (39%) (**Figure 2C** and **Figure 3**), and weak cytoplasmic staining was observed in 13 cases (11%). Twenty-nine cases (24%) of RCC show both membranous staining for RCCma and nuclear reactivity for PAX2 (**Figure 3**). Thirty-two cases (27%) of RCCs fail to show either membranous staining for RCCma or nuclear staining for PAX2 (**Figure 3**). In metastatic RCC, the membranous staining for RCCma was found in 19 cases (20%) and the nuclear staining for PAX2 was demonstrated in 21 of 94 cases (22%) (**Figure 3**). For the six cases of metastatic RCC to the head and neck, only two cases were either positive for RCCma or PAX2. Three cases that metastasize to salivary glands and thyroid were negative for both RCCma and PAX2.

Table 1. Immunoreactivity of RCCma and PAX2 in MEC TMA.

	Case	Site	Grade	RCCma		PAX2	
				Mem	Cyto	Nuc	Cyto
CMEC	5	maxilla	H	-	-	-	++
	7	parotid	IM	-	-	-	+++
	8	palate	L	-	-	-	++
	9	maxilla	L	-	-	-	-
	10	maxilla	L	-	-	-	+
	13	neck	L	-	-	-	+++
	14	maxilla	L	-	-	-	-
	15	parotid	L	-	-	-	+++
	21	bronchus	L	-	-	-	+++
	22	palate	L	+	+	-	+++
	23	palate	L	+	+	-	++
MEC	1	maxilla	H	-	-	-	+
	2	trachea	IM	-	-	-	-
	3	cervical LN	H	-	-	-	-
	4	palate	L	-	-	-	+
	6	parotid	L	-	-	-	++
	11	parotid	IM	-	-	-	+++
	12	parotid	IM	-	-	-	+++
	16	lung	H	-	-	-	++
	17	Palate	L	+	+	-	++
	18	Parotid	L	-	-	-	++
	19	Maxilla	IM	-	-	-	+++
	20	lung	L	-	-	-	++

H: High grade; **IM:** Intermediate grade; **L:** Low grade; **N:** No; **Y:** Yes;
Mem: Membranous; **Cyto:** Cytoplasmic; **Nuc:** Nuclear; **LN:** lymph node

DISCUSSION

MEC is the most common primary tumor of the head and neck with clear cell morphology.[1-5] In our study, almost one-half of MEC contained a variable clear cell component ranging from 20-60% of the tumor, which may pose a challenge in the distinction from the common metastatic CCRCC in the head and neck.[12-18] Two relative sensitive and specific RCC makers, RCCma and PAX2, have become

widely used for aid of diagnosis of both primary and metastatic RCC.[6-10] RCCma antigen is a 200-kd glycoprotein expressed in normal human renal proximal brush border.[19] Although the expression of RCCma antigen has also been reported in normal breast duct and lobules, thyroid follicles and parenchyma of parathyroid glands, only rare non-renal tumors show immunoreactivity to RCCma.[20] In primary RCC, antibody against RCCma has a sensitivity ranging from 20% for sarcomatoid type to 90% for papillary RCC, and 72-84% for CCRCC. The RCCma sensitivity drops to 40-67% for metastatic RCC.[7,8,21] PAX2 is homogenously expressed during the kidney development. Recently, Gokden et al reported that antibody against PAX2 has higher sensitivity and specificity than that of RCCma in metastatic CCRCC. However, the nuclear reactivity of PAX2 has also been shown in tumors from other organs.[10] It is not surprising that neither RCCma nor PAX2 is 100% sensitive or specific for CCRCC.

Our data have shown that specific nuclear PAX2 immunopositivity was found in 39% of primary RCC (47/120). Specific membranous staining pattern for RCCma was noted in 58% of primary RCC cases (69/120). The positive reactivity of PAX2 and RCCma on metastatic RCC is lower than that of primary tumor (22% and 20%, respectively). The positive percentage of the two markers in both primary and metastatic RCC in this study is lower compared to those from previous studies. These could be attributed to several factors, such as higher stringent scoring system, tissue size in TMA and different antibodies used in various studies.

Among six cases of metastatic RCC to head and neck, only two cases (33%) display positivity for either RCCma or PAX2 and none of the three cases that metastasize to salivary glands or thyroid were positive for either PAX2 or RCCma. These findings are interesting and have not been reported previously. Although the percentage of positive cases is small and staining is predominantly in the epidermoid component of the tumor, caution should be made when one interprets positive RCCma results on immunostaining work-up for differential diagnosis between CMEC and metastatic CCRCC. Clinicopathologic correlation combined with a panel of immunohistochemical markers, such as CD10 and vimentin for RCC, CK7 and CK14 for MEC, is essential to render correct diagnosis.

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