The diagnostic value of EMA expression in the renal parenchyma tumors

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Abstract
Renal parenchyma tumors are a heterogeneous group of malignancies that are difficult to diagnose and classify. Immunohistochemistry begun to be routinely used for the diagnosis of these tumors. Panels of antibodies are developed for the diagnostic assessment of these tumors, which include cytokeratins, epithelial membrane antigen and vimentin. Epithelial membrane antigen (EMA) is expressed by most of the tumor cell types. Forty-seven specimens of renal parenchyma tumors were studied immunohistochemically for the expression of EMA. In the majority of the cases, clear cells carcinoma was positive for EMA (25/33, 75.70%). All of the papillary carcinomas were positive, with different staining patterns between the two subtypes. The two cases of chromophobe cells carcinomas were intensely positive with a granular cytoplasmic staining pattern. The mixed epithelial-stromal tumor was negative for EMA in both of the components. Out of the three cases of sarcomatoid carcinomas, one was negative, one was weakly positive (+1) and the last was positive (+2). Intensely positive normal tubes were caught by the tumor proliferation in the negative case and in the negative stained areas of the weakly positive case.

Keywords: EMA, renal parenchyma tumors, immunohistochemistry.

Introduction
Epithelial membrane antigen (EMA) is a fragment of MUC1, referred in most studies as MUC1, a glycoprotein found in human milk fat globule membranes (HMFGP). Since it is a transmembrane glycoprotein packed in the Golgi apparatus, it may have membrane and cytoplasmic (Golgi) staining patterns. It is expresses by a large variety of epithelial cells, mostly of secretory type, such as the luminal cells of the breast secretory units, acinar and ductal cells of the salivary glands, both in normal and pathological conditions [1], mesothelioma cells, in which case it has an important diagnostic role as well, and is positive in ductular reactive liver lesions [2]. It is important not only as a diagnostic marker, but it has roles in tumor cells immune escape and in their migration and metastasis, either by directly affecting the interaction between tumor cells and immune cells, or by induction of immunological tolerance. It is correlated with the number of myofibroblasts in oral cancer, suggesting a role in the communication between tumor and stroma [3], whereas its expression in colorectal carcinoma is associated with poor prognosis [4]. Its overexpression is regulated by PPARγ in trophoblast cells [5]. In renal cells carcinomas, the epithelial membrane antigen is a constant presence in the immunohistochemical diagnostic panel, as it is normally positive in conventional renal carcinoma, aside with low molecular weight cytokeratins, CK18, pan-cytokeratin and vimentin [6]. The epithelial membrane antigen is also positive in rhabdoid cells, along with vimentin and cytokeratin [7]. It is a useful marker in distinguishing renal cell carcinoma from other retroperitoneal tumors such as retroperitoneal paraganglioma, and adrenal cortical lesions, particularly in small tissue samples [8].

We have studied their expression in the normal remaining renal parenchyma of patients with kidney tumors.

Materials and Methods
Forty-seven renal parenchyma tumors specimens from patients admitted between 1999 and 2004 were selected from the archive of the Urology Clinic of the Emergency County Hospital, Timișoara, were primary processed, performed morphological diagnosis and pretreated for immunohistochemistry. Briefly, the specimens were fixed in 4% buffered formalin, embedded in paraffin and sectioned at 3–5 µm. The slides were then dewaxed and rehydrated in baths of benzene and then ethylic alcohol with decreasing concentration, and then antigen retrieval was performed by microwave heating at pH 6 before blocking the endogenous peroxidase. The slides were then dewaxed and rehydrated and either stained with the usual Hematoxylin–Eosin staining for the morphological diagnosis or pretreated for immunohistochemistry. Morphological diagnosis was performed on Hematoxylin–Eosin stained slides, according to WHO classification. Additional slides were immunohistochemically stained with the polyclonal anti-EMA antibody. Briefly, slides were dewaxed and rehydrated in baths of benzene and then ethyl alcohol with decreasing concentration, and then antigen retrieval was performed by microwave heating at pH 6 before blocking the endogenous peroxidase. Then, the primary antibody and LSAB2 working systems were applied, followed by nuclear counterstaining with Lillie’s Hematoxylin. The slides were then examined at a Nikon i80 microscope, with the tubular system of the nephron.
and collecting tubes as internal positive control. The assessment of the staining was by a positive cells number score, as following: 0 for the negative cases, +1 for the cases with few isolated or small groups of positive cells, +2 for the cases with less than 33% positive cells, +3 for the specimens with positive cells between 33% and 66%, and +4 for the tumors with more than 66% positive cells. The positive cells had a homogenous membrane staining pattern. After staining, the slides were dehydrated and mounted with Canada balsam.

Statistic data analysis was performed with SPSS software package, version 17.

**Results**

**Epithelial membrane antigen expression (EMA) in normal renal parenchyma**

The internal positive control of EMA immunostaining was represented by the distal tubules, intermediate segments and collecting tubes from the normal adjacent renal parenchyma and by the urothelium. In the tubular system, the final reaction product was cytoplasmic, with granular pattern and high intensity (Figure 1, a and b). For the urothelium, the reaction product was positive in the cells from the superficial area, but with membrane pattern (Figure 2).

**Expression of EMA in renal parenchyma tumors**

The distribution of the final reaction product was different from one tumor to another, with two staining patterns. The classical membrane homogenous, high intensity staining pattern was noticed in many cases (Figure 3a). The second staining pattern was diffuse granular cytoplasmic staining pattern (Figure 3b).

The reaction was sometimes heterogeneous, characterized by the presence of intensely or moderately stained cells, alternating with negative cells (Figure 4a). The rarest staining pattern noticed was the one with isolated positive cells isolated in a mass of negative tumor cells (Figure 4b).

In the majority of the cases, clear cells carcinoma was positive for EMA (25/33, 75.70%). In negative cases, no tumor cell was positive, although normal tubules caught in the tumor proliferation were intensely positive (Figure 5).
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The intensity of the staining and the number of positive cells were correlated with the differentiation grade of the tumors. Most of the tumors with a Fuhrman 1 and 2 score had a low number of positive cells, with membrane staining pattern (Figure 6a). In tumors with cystic transformation, the cells lining the cystic cavity were positive, stained at the apical pole (Figure 6b). In tumors with a Fuhrman score of 3, the reaction was intense, with granular cytoplasmic staining pattern (Figure 6c).

The classic staining pattern for epithelial membrane antigen in conventional renal carcinomas was of membrane, homogenous, with high intensity staining (Figure 7).

All of the papillary carcinomas were positive for EMA, but the intensity of the staining and the distribution of positive cells were different, according to the subtype. In type I papillary carcinomas, most of the cells were positive with membrane pattern, predominantly at the apical pole (Figure 8a). In type II papillary carcinoma, the reaction was intense and diffuse, both with membrane and cytoplasmic pattern (Figure 8b). EMA immunostaining may allow for the differential diagnosis between papillary carcinoma subtypes. On the other hand, compared with AE1/AE3 cytokeratin, the intensity of the staining was lower in the case of subtype I of papillary carcinoma.

The two cases of chromophobe cells carcinomas were intensely positive. The staining pattern was granular cytoplasmic, without membrane intensifying (Figure 9a). Only a part of the chromophobe cell population still had the perinuclear halo, most of the cells being intensely positive. In one of the cases, we have noticed a heterogeneous staining pattern, with most of the cells intensely positive, admixed with pale stained cells (Figure 9b).

The mixed epithelial-stromal tumor was negative for EMA in both of the components. Out of the three cases of sarcomatoid carcinomas, one was negative, one was weakly positive (+1) and the last was positive (+2). In the case with +1 score, the positive cells were isolated and surrounded by negative cells (Figure 10a). In the positive case (+2), the positive cells were distributed focally, in small groups or isolated, representing less than 33% of the tumor cells (Figure 10b).

Intensely positive normal tubes were caught by the tumor proliferation in the negative case and in the negative stained areas of the weakly positive case (Figure 11).
Figure 6a – Clear cells carcinoma. Microcystic type with positive cells lining the lumen. EMA immunostaining, ×400.

Figure 6b – Clear cells carcinoma. Microcystic type with positive cells lining the lumen. Homogenous granular cytoplasmic staining pattern. EMA immunostaining, ×400.

Figure 6c – Clear cells carcinoma. Membrane pattern. EMA immunostaining, ×400.

Figure 7 – Clear cells carcinoma. Intensely stained cells, scored +4. EMA immunostaining, ×400.

Figure 8a – Papillary carcinoma. Subtype I, with predominantly apical staining. EMA immunostaining, ×400.

Figure 8b – Papillary carcinoma. Subtype II, with diffuse cytoplasmic pattern. EMA immunostaining, ×400.
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Figure 9a – Chromophobe cells carcinoma. Intensely granular cytoplasmic staining pattern. EMA immunostaining, ×400.

Figure 9b – Chromophobe cells carcinoma. Heterogeneous staining pattern with intensely and pale stained cells. EMA immunostaining, ×400.

Figure 10a – Sarcomatoid carcinoma with a single positive cell. EMA immunostaining, ×400.

Figure 10b – Sarcomatoid carcinoma. Focal intense positive staining in sarcomatoid carcinoma. EMA immunostaining, ×400.

Figure 11 – Negative sarcomatoid carcinoma, with intensely positive tubes caught in the proliferation. EMA immunostaining, ×400.

Discussion

The EMA immunostaining was positive in 37 of the 47 cases of renal parenchyma tumors (78.70%). The staining was more intense in the tumors with high Fuhrman score. The differential diagnosis of the chromophobe cells carcinomas involves the use of immunohistochemistry with an antibody panel that must include cytokeratins, epithelial membrane antigen (EMA) and vimentin.

The literature’s data suggest that type I papillary carcinomas have an immunohistochemical profile similar to the distal tubules, being positive for EMA and cytokeratin 7, as well as for vimentin [10, 11].

Although in our study all of the tumors were positive, the staining pattern was different between the two papillary carcinoma subtypes. An immunohistochemical study on 21 cases of chromophobic cells...
carcinomas compared with clear cells carcinomas and renal oncocytes [12] revealed the staining of the chromophobe cells with EMA and their negative staining for vimentin.

The cytoplasmic staining pattern for EMA was present in most of the chromophobic cells carcinomas, which is in concordance with the data from the literature, in which the ratio of positive cases was 98% [13]. The same study documented a positive ratio for EMA of only 57% of the cases of papillary carcinomas, while we have noticed that all of the cases were positive for EMA.

Another study on pediatric renal tumors showed a significant degree of EMA expression in Wilms, rhabdoid and clear cells tumors [14].

The diagnostic role of EMA in renal cell carcinoma is also maintained in metastatic tumors, especially those in the adrenal gland. The primary tumors of the adrenal gland were negative to the marker in one study, while 78% out of the metastases were positive to EMA/MUC1 [15].

Moreover, EMA/MUC1 might be useful in the diagnosis of rare tumors, both of the renal tissue, or in other locations, such as pituitary tumor [16], or renal small cells oncocytoma [17] or rhabdoid morphology adult renal cell carcinoma [18].

Some cutaneous tumors, such as the sebaceous carcinoma, might be positive for EMA, and therefore the marker should be included in the primary diagnostic panel [19].

The expression of EMA/MUC1 may even be a diagnostic pitfall in some of the tumors of mesenchymal origin, such as Ewing sarcoma and epithelioid fibrous histiocytoma, where the cells might be positive, without being significant for an epithelial tumor [20, 21].

Finally, EMA expression may be useful to differentiate not only between different types of tumors, but also between tumor and reactive cells, along with other markers, in pleural cytology [22].

The role of EMA/MUC1 in tumor pathology is still unclear, even if some studies correlate its expression with overall survival of the patients in renal cell carcinoma [23] and in other types of tumors [24].

Most of the antiproliferative actions of MUC1/EMA are considered to be related to its binding to β-catenin [25] or to β-catenin and EGFR, in which case it inhibits proliferation, migration and invasion of tumour cells in an experimental setting [26]. There is one study that demonstrates the ability of MUC1 to inhibit the activation of caspase 8 by binding to p18 close to the catalytic segment [27]. The role of MUC1/EMA in cell survival was also used as a therapeutic approach in mammary carcinoma cell lines by activation of NK cells targeted to MUC1 expressing cells [28] or by targeting the intracytoplasmic domain and therefore inhibiting proliferation [29].

Significant amount of data, however, document the role of MUC1 in tumor progression by several mechanisms, either by direct stimulation of hyperplasia [30], by having the tumor cells depending on MUC1 signaling for survival [31], or by upregulating matrix metalloproteinases [32], or by enhancing tumor angiogenic response by stimulating the Akt pathway in experimental breast cancer [33].

It may act as an inhibitor of terminal differentiation in some human leukemia cells [34], as well as a modulator of hypoxic response [35] in conjunction with the angiogenic activity mentioned earlier [33].

However, the main role of EMA/MUC1 immunostaining in kidney parenchyma tumors remains the diagnostic one, especially in chromophenic renal cell tumors, which express EMA in 75–100% of the cases [36].

Conclusions

EMA immunostaining was positive in 37 of the 47 cases of renal parenchyma tumors (78.70%). In the papillary carcinoma subtypes, EMA had different distribution patterns. The staining score of the tumors correlated with the nuclear grade. EMA was present in most of the chromophobic cells carcinomas.

References


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