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Original Article

Immunohistochemical expression of tumor antigens MAGE-A3/4 and NY-ESO-1 in renal oncocytoma and chromophobe renal cell carcinoma

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SUMMARY

The distinction between renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC), especially the eosinophilic variant, can often be difficult. Our study has documented for the first time the expression of MAGE-A3/4 and NY-ESO-1 cancer testis antigens (CTAs) in these tumors. A total of 35 patients (17 ROs and 18 ChRCCs) were included in the study. Two antibodies were used for immunohistochemical staining: 57B recognizing multiple MAGE-A and D8.38 recognizing NY-ESO-1 CTAs. Fifteen (88.2%) samples of RO stained positively for both MAGE-A3/4 and NY-ESO-1 antigens. Regarding ChRCC, seven (38.9%) stained positively for MAGE-A3/4 and six (33.3%) for NY-ESO-1 antigens. Median MAGE-A3/4 expression was moderately positive in RO and negative in ChRCC. The difference in MAGE-A3/4 expression between two tumor groups was significant ($P = 0.0013$). Median NY-ESO-1 expression was strongly positive in RO and negative in ChRCC. The difference in NY-ESO-1 expression between two tumor groups was also significant ($P = 0.0008$). Our study has shown that RO had a significantly higher expression of both CTAs. However, additional research is needed to clarify their potential diagnostic implications.

Introduction

Renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC) are both renal epithelial neoplasms thought to arise from intercalated cells of collecting ducts. Together they account for approximately 10% of surgically removed renal epithelial tumors [4]. Oncocytomas are benign, non-encapsulated neoplasms composed of round-to-polygonal cells with densely granular eosinophilic cytoplasm (so-called oncocytes), which form compact nests, acini, tubules, or microcysts. Oncocytomas occasionally have sclerosed central area [4,14]. ChRCC are solid tumors made up of large polygonal cells with prominent cell membranes, pale cytoplasm, and usually a perinuclear halo. They include three subtypes: classic, eosinophilic, and mixed [24]. The majority of ChRCCs are stage T1 and T2, and only a few cases of lymph node and distant metastasis have been described [4].

The distinction between RO and ChRCC, especially its eosinophilic variant, can sometimes be difficult due to their overlapping morphological characteristics. Histology, ultrastructural examination, and staining with Hale's colloidal iron can be used for their differentiation in daily practice. In recent years, there have been attempts to find an immunohistochemical marker that could also help in diagnostics [1,5,15–17,19].

Cancer testis antigens (CTAs) comprise a family of more than 40 genes expressed in a wide variety of malignant tumors [21]. In normal tissue, their expression is mostly limited to germ cell lines. Because of their ability to induce immune responses, CTAs are being evaluated as targets for therapeutic cancer vaccines [3,23].

There is only limited information available on the expression of CTAs in different histological subtypes of renal tumors [18,25].

The aim of this study was to investigate the immunohistochemical expression of MAGE-A3/4 and NY-ESO-1 CTAs in ROs and ChRCCs. To our knowledge, there are no studies regarding the immunohistochemical expression of these CTAs in RO and/or ChRCC.

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Table 1
Clinicopathologic characteristics and results of immunohistochemical staining for MAGE-A3/4 and NY-ESO-1 in renal oncocytoma.

No.	Age	Gender	Tumor size (cm)	MAGE-A3/4[a]	NY-ESO-1[a]
1.	80	F	6.0	+	++
2.	47	M	4.3	–	–
3.	74	F	2.6	++	++
4.	52	M	6.0	++	+++
5.	62	M	3.5	+	+
6.	69	F	2.0	+++	+++
7.	66	M	3.0	–	–
8.	58	F	4.2	+	+
9.	61	F	1.7	+++	+++
10.	62	F	3.0	++	+++
11.	57	M	4.0	+++	+++
12.	77	M	0.9	+++	+++
13.	68	F	2.2	+++	+++
14.	68	M	3.0	++	+++
15.	69	F	6.5	+++	+++
16.	55	F	8.0	++	++
17.	70	F	1.5	+++	+++

[a] (–) = No staining in tumor cells; (+) up to 10% of tumor cells positive; (++) >10–50% of tumor cells positive; (+++) more than 50% of tumor cells positive.

Materials and methods

Tissue samples

Pathology reports of histologically confirmed ROs and ChRCCs diagnosed at two Departments of Pathology (Ljudevit Jurak University Department of Pathology, Sestre Milosrdnice University Hospital, and Department of Pathology, University Hospital Dubrava, Zagreb) were reviewed. The diagnosis of all cases was established according to the criteria set forth in the 2004 WHO Classification of Tumors of the Urinary System and Male Genital Organs [4]. There were 35 cases in total: 17 ROs and 18 ChRCCs. Among patients with RO, 10 were females and 7 males. Patients' age ranged from 47 to 80 years (mean 64.4). Tumor size ranged from 0.9 to 8 cm (mean 3.7 cm). Among patients with ChRCC, 11 were females and 7 males. Patients' age ranged from 34 to 76 years (mean 58.2). Tumor size ranged from 1.7 to 17 cm (mean 7.6).

Immunohistochemistry

Two antibodies were used for immunohistochemical staining. 57B was generated on immunization of mice with recombinant MAGE-A3 [13]. However, this antibody recognizes a variety of MAGE-A molecules, and it is currently considered a multi-MAGE-A-specific reagent [11]. D8.38 antibody, recognizing NY-ESO-1 and its homologous LAGE-1 CTA, has been previously described [22].

Tissue sections of 3–5 μm thickness were cut from paraffin-embedded tissue blocks, placed on object slides (Menzel-Glaser, Germany), and incubated for 20 min in a thermostat at 60 °C.

The sections were then deparaffinized and incubated for 3× 5 min in 10 mmol/L of citrate buffer (pH 6.0) in a microwave oven at 800 W. Subsequently, tissue slides were washed with phosphate-buffered saline (PBS) buffer (pH 7.2), and endogenous peroxidase activity was blocked by a 5-min treatment with hydrogen peroxide (Dako, No. S2023). Slides were then washed with PBS-buffer and incubated for 90 min with MAGE-A3/4 57B or NY-ESO-1 D8.38 undiluted supernatants at room temperature.

After washing in PBS, the secondary biotinylated antibody (DAKO, No. K0690) was added for 30 min of incubation. Slides were then washed with PBS-buffer and treated with streptavidin–horseradish peroxidase (Dako, No. K0690) for 30 min. Tissue sections were washed once more in PBS-buffer, and then Chromogen (Dako, No. K3468) was added for 5 min. Slides were washed in distilled water, stained with hemalaun (Dako, No. S2020) for 1 min, washed with water, dehydrated with alcohol (96%), cleared with xylene, and mechanically covered.

Melanoma and testicular tissues expressing CTAs were used as positive controls. As a negative control, we replaced primary antibodies with isotype matched immunoglobulins.

The results of the immunohistochemical staining were expressed semiquantitatively as follows: negative response (–): no staining in tumor cells; weakly positive response (+): up to 10% of tumor cells positive; moderately positive response (++): >10–50% of tumor cells positive; and strongly positive response (+++): more than 50% of tumor cells positive.

Statistical analysis

Statistical analysis was done using Mann–Whitney and Spearman's rank correlation tests. $P < 0.05$ was considered to be statistically significant.

Results

Clinical and histological data and results of immunohistochemical staining are summarized in Tables 1 and 2. Fifteen ROs (88.2%) were positive for both MAGE-A3/4 and NY-ESO-1 antigens (Fig. 1A and B). Regarding ChRCC, 7 (38.9%) showed a positive reaction for MAGE-A3/4 antigen and 6 (33.3%) for NY-ESO-1 antigen (Fig. 1C and D). Median MAGE-A3/4 expression was moderately positive in ROs and negative in ChRCCs. The difference in MAGE-A3/4 expression between two tumor groups was statistically significant ($P = 0.0013$). Median NY-ESO-1 expression was strongly positive in ROs and negative in ChRCCs. The difference in NY-ESO-1 expression between two tumor groups was also significant ($P = 0.0008$). The pattern of staining was diffuse cytoplasmic. Comparison of the expression of MAGE-A3/4 and NY-ESO1 antigens with the nuclear grade in ChRCC showed no statistically significant correlation ($P = 0.9$ and $P = 0.7$, respectively). Also the size of ChRCC and the expression of MAGE-A3/4 and NY-ESO1 did not correlate significantly ($P = 0.4$ and $P = 0.6$, respectively).

Discussion

Renal oncocytoma and ChRCC, especially its eosinophilic variant, can often be confused with one another due to their similar morphology. The distinction between these tumors is clinically relevant because they have different biological courses; RO is a benign neoplasm, whereas ChRCC has malignant potential, particularly its sarcomatoid variant which is associated with more aggressive tumor behavior [4].