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Apoptotic markers (P53, Bcl-2 and Bax) expression in renal oncocytoma and chromophobe renal cell carcinoma

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Abstract

Background and Purpose: *Renal oncocytoma (RO) and eosinophilic variant of chromophobe renal cell carcinoma (ChRCC) have overlapping morphologic, immunohistochemical, histochemical and ultrastructural features. Their distinction is mandatory since RO is a benign tumor, while ChRCC is a low-grade malignant tumor which has metastatic potential and may undergo sarcomatoid transformation. The aim of this study was to determine expression of P53, Bcl-2 and Bax in ROs and ChRCCs and to explore whether these markers could be useful in differential diagnosis of RO and ChRCC.*

Patients and Methods: *We analyzed 61 cases (28 ChRCCs and 33 ROs) by immunohistochemistry using primary antibodies to P53, Bcl-2 and Bax. The staining percentage and staining intensity scores were multiplied to give immunohistochemical staining index (ISI) .*

Results and Conclusion: *All specimens showed positive reaction for Bcl-2 and Bax. There was no significant difference in the ISI for Bcl-2 and P53. Statistical analysis showed significant difference in the ISI of Bax between ROs and ChRCCs. Moreover, cases of ROs had cytoplasmic pattern of reaction for Bax, while the pattern of reaction for Bax in cases of ChRCCs was membranous.*

Further studies are needed to confirm the possible use of Bax immunostaining in differential diagnosis of RO and ChRCC.

INTRODUCTION

Chromophobe renal cell carcinoma (ChRCC) is a low-grade malignant neoplasm which accounts for 5% of renal epithelial tumors. Two main variants are classic and eosinophilic. ChRCC has a metastatic potential and may undergo sarcomatoid transformation, which is associated with more aggressive behavior *(1).* Renal oncocytoma (RO) is a benign tumor which comprises approximately 5% of all primary renal tumors *(1).* The distinction between RO and ChRCC is very important due to their different biological behavior. Overlapping morphological features of RO and eosinophilic variant of ChRCC, can often complicate making a correct diagnosis.

P53 is known inducer of apoptosis and it is the most common genetic mutation found in cancers. The Bcl-2, in contrast, prevents apoptosis and has many interactions, especially with Bax, which acts to promote cell death *(2)*.

There have been numerous studies that explored the possible use of various immunohistochemical markers in differential diagnosis of RO and ChRCC, but there is currently no reliable immunohistochemical marker for this purpose. Our recent work revealed higher number of apoptotic cells in the group of ROs compared to the group of ChRCCs (Round table – Apoptosis, programmed cell death, Zagreb, 2010).

The aim of this study was to determine expression of P53, Bcl-2 and Bax in ROs and ChRCCs and to explore whether these markers could be useful in differential diagnosis of RO and ChRCC.

PATIENTS AND METHODS

The files from the University Department of Pathology, Sestre milosrdnice University Hospital Centre, Zagreb, from the period 1999-2010, were searched for cases of histologically confirmed ChRCC and RO. There were 61 cases in total (28 ChRCCs and 33 ROs). Among patients with ChRCC, 14 were females, and 14 males. Patients' age ranged from 31-82 years (mean 59.4). Tumor size ranged from 0.7-17 cm (mean 6.2). Among patients with RO, 18 were females, and 15 males. Patients' age ranged from 46-80 years (mean 63.9). Tumor size ranged from 0.9-8 cm (mean 3.4). All specimens were routinely fixed in 10% buffered formalin, embedded in paraffin, cut at 5-µm thickness and stained with haematoxylin and eosin. All cases were reviewed and the diagnosis of RO and ChRCC was established according to the criteria proposed by 2004 WHO for classification of renal tumors *(3)*. Immunohistochemistry was performed by Microwave Streptavidine Immunoperoxidase protocol (MSIP) in DAKO TechMate Horizon automated immunostainer. We used primary antibodies to P53 (Clone DO-7, Dako), Bcl-2 (Clone 124, Dako) and Bax (Clone A3533, Dako). Dilution of antibodies for P53 and Bcl-2 was „ready to use". Antibody for Bax was diluted 1:500. Positive control for P53 staining was colon adenocarcinoma tissue and for Bax and Bcl-2 staining breast carcinoma tissue. Immunohistochemical analysis for P53 and Bcl-2 was performed on all specimens and the immunohistochemical analysis for Bax was performed on 20 specimens (10 ROs and 10 ChRCCs). Immunohistochemical reaction was evaluated semiquantitatively on 1000 tumor cells and the percentage of positive cells as well as staining intensity were scored on a scale 0-3. The percentage of positive cells was designated as: 0 = no positive cells; 1 = 1-10% positive cells; 2 = >10-25% positive cells, 3 = more than 25% positive cells. Staining intensity was labelled as: 0 = no staining, 1 = weak staining; 2 = moderate staining; 3 = strong staining. The staining percentage and staining intensity scores were multiplied to give immunohistochemical staining index (ISI) for each case. According to literature data, four groups are formed: ISI 0 = no reaction; ISI 1 = weak reaction (1,2,3); ISI 2 = moderate reaction (4 or 6); ISI 3 = strong reaction (9) *(4)*. Statistical analysis was performed using the Mann-Whitney *U*-test and χ^2-test. The level of significance was set at **p=0.05.**

RESULTS

Immunohistochemical staining index for P53, Bcl-2 and Bax is shown in Table 1.

All specimens showed positive reaction for Bcl-2 with predominantely moderate reaction (ISI 2). Statistical analysis did not show significant difference in the ISI of Bcl-2 between ROs and ChRCCs. (p=0.315)

Immunohistochemical reaction for P53 was mostly weak (ISI 1) and 7 cases in both groups of tumors had ISI 0. Statistical analysis of the P53 expression between ROs and ChRCCs did not show significant difference**.** (p=0.682)

All 20 specimens that were analyzed for the Bax expression showed positive reaction for this marker. The majority of ROs had ISI 3. Immunohistochemical reaction for Bax in the group of ChRCCs was predominan-

TABLE 1

Immunohistochemical staining index (ISI) for P53, Bcl-2 and Bax in renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC).

	P53		Bcl-2		Bax	
	RO (n=33)	ChRCC (n=28)	RO (n=33)	ChRCC (n=28)	RO (n=10)	ChRCC (n=10)
ISI 0	7 (21.2%)	7 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
ISI 1	23 (69.7%)	19 (67.9%)	2 (6.1%)	4 (14.3%)	1 (10%)	4 (40%)
ISI 2	3 (9.1%)	2 (7.1%)	23 (69.7%)	19 (67.9%)	3 (30%)	5 (50%)
ISI 3	0 (0%)	0 (0%)	8 (24.2%)	5 (17.8%)	6 (60%)	1 (10%)

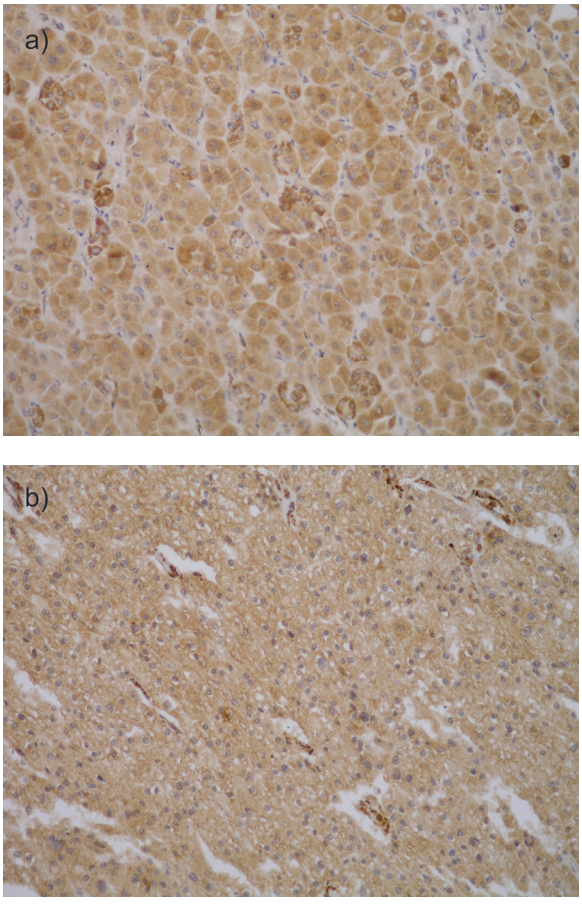

Figure 1. *Immunostaining for Bax: a) cytoplasmic pattern of reaction in renal oncocytoma (X200); b) membranous pattern of reaction in chromophobe renal cell carcinoma (X200).*

tely weak or moderate (ISI 1 and ISI 2). Statistical analysis showed signiffcantly higher ISI of Bax in ROs compared to ChRCCs. (p=0.022) Moreover, cases of ROs had cytoplasmic pattern of reaction for Bax, and the pattern of reaction for Bax in cases of ChRCCs was membranous (Figure 1).

DISCUSSION

Distinction between RO and eosinophilic variant ChRCC is common diagnostic problem in urologic pathology. ROs and eosinophilic variant of ChRCCs have overlapping morphologic, immunohistochemical, histochemical and ultrastructural features. Their distinction is mandatory since they have different biological courses. ChRCC has malignant potential, especially its sarcomatoid variant, whereas RO is a benign tumor *(3).* Numerous studies explored the possible use of many immunohistochemical markers in differential diagnosis of RO and ChRCC such as caveolin-1, CD63, anti-mitochondrial antibody, cytokeratin 14, cytokeratin 7 and 20, vimentin, glutathione S-transferase α, CD10, CD117, claudin-7, claudin-8, kidney-specific cadherin, p53, progesterone, MAGE and NY-ESO *(5-11).* Antimitochondrial antibodies, parvalbumin and BCA2 are considered to be good markers for differentiating between these tumors *(12-15).* However, there is currently no specific immunohistochemical marker for differential diagnosis of these tumors.

Few authors analyzed the immunohistochemical expression of Bcl-2 and Bax in the clear cell renal cell carcinoma (CCRCC) with respect to established prognostic factors for CCRCC. Vasavada *et al*. found the Bcl-2 and Bax expression to be correlated with higher tumor grade but none of the other factors (tumor recurrence, metastasis, survival rate) *(16).* In the other study the Bcl-2 expression also did not correlate with disease-free or overall survival. However, this group of authors found that the Bcl-2 expression occurred more frequently in matched patient metastases when compared to primary tumors *(17).*

The tumor suppressor gene p53 is essential in maintaining the genomic integrity of cells. It is involved in controlling a checkpoint during the G1 phase of the cell cycle and induces cell cycle arrest and apoptosis in the presence of damaged DNA *(18).* Mutation of p53 has been reported in approximately 50% of all human cancers, although these mutations generally occur late in tumorigenesis process *(19, 20).* The reported range of P53 overexpression in CCRCC varies from 0% to 40% *(16, 21-27).*

We analyzed the immunohistochemical expression of apoptosis-related markers P53, Bcl-2 and Bax in ROs and ChRCCs. All specimens showed positive reaction for Bcl-2 and Bax. There was no significant difference in the ISI for Bcl-2 and P53. The results regarding Bax expression showed significant difference in the pattern of reaction and ISI between the two tumor groups, but number of cases is insufficient to make final conclusions.

Further studies are needed to confirm the possible use of Bax immunostaining in differential diagnosis of RO and ChRCC.

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