THE VALUE OF CASPASE 3 IN DIFFERENTIATING RENAL ONCOCYTOMA FROM CHROMOPHOBE RENAL CELL CARCINOMA

FRAN ČAKAR[1] and TIHANA REGOVIĆ DŽOMBETA[1,2]

[1]University of Zagreb, School of Medicine, Department of Pathology and [2]Sestre milosrdnice University Hospital Centre, Ljudevit Jurak Department of Pathology and Cytology, Zagreb, Croatia

Aim: Renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC) are epithelial neoplasms of the kidney with overlapping histologic features, making their differential diagnosis one of the pitfalls in renal pathology. ***The aim*** of this study was to assess the level of caspase 3 expression in RO and ChRCC, in order to investigate its potential value in their differentiation. ***Methods:*** The study included 24 RO cases and 24 ChRCC cases, taken from the archives of Ljudevit Jurak Department of Pathology and Cytology, Sestre milosrdnice University Hospital Centre Zagreb. The results of immunohistochemical analysis were determined semiquantitatively using immunohistochemical staining index (ISI). Statistical analysis was done using Mann-Whitney U test. ***Results:*** All samples showed positive immunohistochemical reaction for caspase 3, with the majority of RO showing moderate ISI and the majority of ChRCC showing low ISI. ***Conclusion:*** The use of caspase-3 could favor the diagnosis of RO in cases where the tumor shows strong and diffuse staining. Further studies on a larger number of cases are needed to confirm our results.

Key words: caspase 3, renal oncocytoma, chromophobe renal cell carcinoma, immunohistochemistry

Address for correspondence: Tihana Regović Džombeta, MD, PhD
Department of Pathology
School of Medicine, University of Zagreb
Šalata 10
10 000 Zagreb, Croatia
Tel: 01 4566 978: e-mail: tihana.dzombeta@mef.hr

INTRODUCTION

Renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC) are epithelial neoplasms of the kidney that share many histologic similarities. Differential diagnosis between the two is often difficult due to their overlapping features, making it one of the pitfalls in renal pathology.

Oncocytoma is a benign neoplasm of the kidney which accounts for 5%-9% of all renal neoplasms (1). It can occur over a wide age range, but peaks in the seventh decade of life, and is usually found incidentally (2). Grossly, RO is usually unencapsulated and well circumscribed. Microscopically, it consists of large, uniform cells having abundant eosinophilic cytoplasm due to the high number of mitochondria. The cells are arranged in nested or tubulocystic pattern and embedded in hyalinized or myxoid stroma.

Chromophobe renal cell carcinoma is in epidemiological term very similar to RO; it accounts for 5%-7% of all renal carcinomas, occurs over a wide age range but peaks in the sixth decade, and is often found incidentally (1). It is grossly well circumscribed, usually demarcated by a fibrous capsule (3). Microscopically, it is typically arranged in solid sheets separated by often hyalinized vascular septa. There are two types of tumor cells, i.e. the large, pale ones with distinct cell membranes, located peripherally within the sheets, and the smaller ones with eosinophilic, granular cytoplasm, usually located centrally (4). Unlike RO, the cytoplasm of ChRCC cells is ultrastructurally crowded with loose glycogen deposits and not mitochondria (1).

A number of studies investigated histologic features, the use of colloidal iron, immunohistochemical markers or ultrastructural characteristics that could potentially be used for differentiating these tumors (3-7).

Due to the high content of mitochondria in RO, one of the potential differences could be the expression of apoptotic markers such as caspase 3, an endoprotease involved in terminal phase of apoptosis, degradation of the DNA molecule.

AIM

The aim of this study was to assess the level of caspase 3 expression in RO and ChRCC, in order to investigate its potential value in their differentiation.

MATERIALS AND METHODS

Materials

The study included 24 RO cases and 24 ChRCC cases taken from the archives of Ljudevit Jurak Department of Pathology and Cytology, Sestre milosrdnice University Hospital Centre, Zagreb (Figure 1).

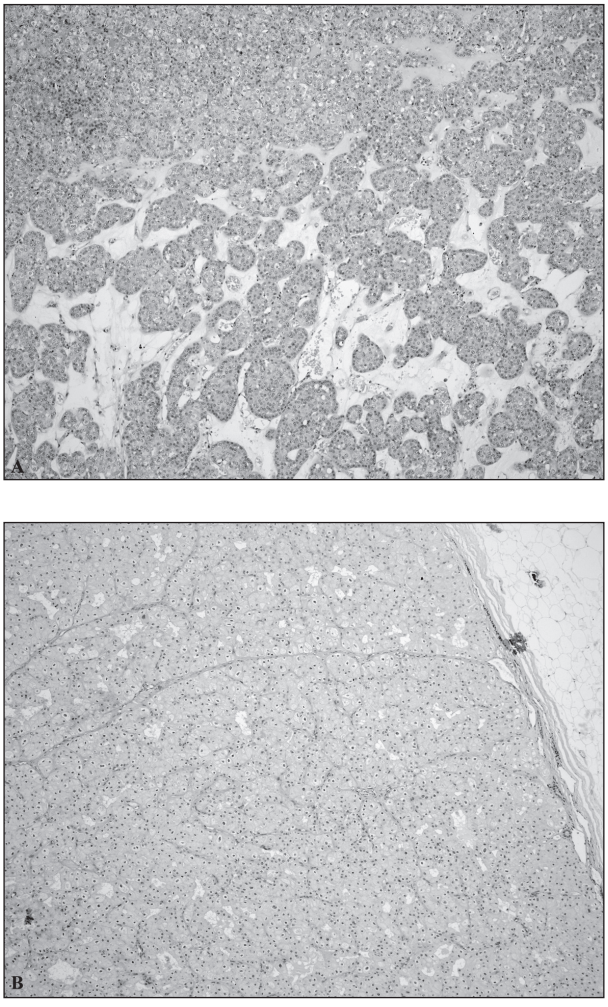

Fig. 1. *Histologic characteristics of oncocytoma (A, HE x100) and chromophobe renal cell carcinoma (B, HE x100).*

Oncocytoma cases were diagnosed in 11 female and 13 male patients aged 46-82 (mean age 65) years. Tumor size ranged from 1.5 to 7 (mean 3.1) cm.

The ChRCC cases were found in 12 female and 12 male patients aged 26-76 (mean age 52) years. Tumor size ranged from 2 to 23 (mean 7.2) cm. Sarcomatoid transformation was found and histologically verified in 2 ChRCC cases. One patient also had 2 oncocytomas beside ChRCC.

Immunohistochemistry

Immunohistochemical staining was performed using standard procedures on a DAKO TechMate Horizon automated immunostainer (DAKO, Copenhagen, Denmark). The pretreatment of sections was performed using Dako PT link (deparaffinization, rehydration and epitope retrieval). After blocking the endogenous peroxidase activity by 5-minute incubation with 3% hydrogen peroxide, the sections were incubated at room temperature with primary polyclonal rabbit antibody against caspase-3 (code ab13847, Abcam, dilution 1:50) for 30 minutes. This was followed by incubation with the labeled polymer (EnVision HRP, DAKO, Dennmark). Color was developed by incubation with 3,3'-diaminobenzidine tetrahydrochloride and slides were counterstained by hematoxylin. Tonsil was used as a positive control.

The results of immunohistochemical analysis were determined semiquantitatively using immunohistochemical staining index (ISI), obtained by multiplying the percentage of positive cells (PPC) and staining intensity (SI), as previously described (8). The PPC was scored as 0 for no positive cells, 1 for up to 10% positive cells, 2 for >10%-50% positive cells and 3 for more than 50% positive cells, while SI was scored as 0 for no staining, 1 for weak staining, 2 for moderate staining and 3 for strong staining. The ISI was labeled as follows: 0=zero; 1-3=low; 4-6=moderate; and 9=high.

Statistical methods

Statistical analysis was done using Mann-Whitney U test; $p<0.05$ was considered to be statistically significant. The analysis was performed using IBM SPSS Statistics software version 21.0.

RESULTS

The results of immunohistochemical analysis are summarized in Table 1. All samples showed positive immunohistochemical reaction for caspase 3, with the majority of ROs showing moderate ISI and the majority of ChRCCs showing low ISI. Not a single ChRCC showed high ISI, while 6 (25%) RO cases showed high ISI. Only 2 (8,3%) RO cases showed low ISI. Figure 2 shows expression of caspase 3 in RO and ChRCC.

Table 1
Caspase 3 expression in renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC), shown as immunohistochemical staining index (ISI)

	Negative	Low	Moderate	High
Oncocytoma	0	2 (8,3%)	16 (66,7%)	6 (25%)
Chromophobe renal cell carcinoma	0	14 (58,3%)	10 (41,7%)	0

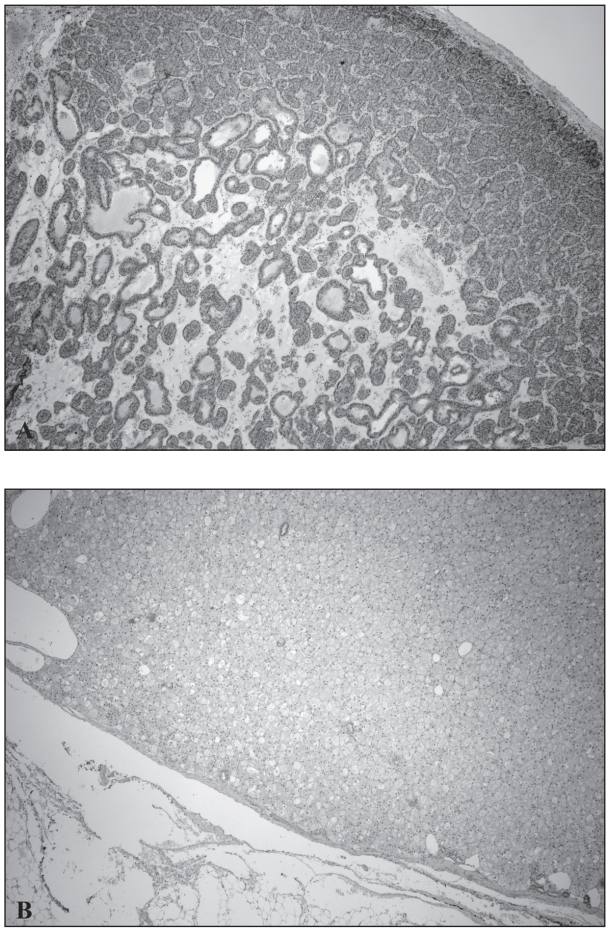

Fig. 2. *Immunohistochemical expression of caspase 3 in renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC): (A) microphotograph showing high immunohistochemical staining index (ISI 9) in RO tissue sample (x100); (B) microphotograph showing moderate immunohistochemical staining index (ISI 6) in ChRCC tissue sample (x100).*

The difference in immunohistochemical staining index of caspase 3 between the tumors was statistically significant (Mann-Whitney U test; $p<0,01$). Renal oncocytomas showed a mean ISI value of 6,46, while the mean ISI in ChRCCs was 3,92.

Also, we found a statistically significant difference between the age of patients with ROs and ChRCCs, with the patients having RO being on average around a decade older than the ones with ChRCC.

DISCUSSION

Oncocytoma and ChRCC are epithelial tumors thought to originate from the intercalated cells of collecting ducts of the kidney. They both appear more often sporadically (around 90% of cases), with approximately 10% of the cases occurring as part of a syndrome, usually the same one, with the same genetic mutations, such as Birt-Hogg-Dubé syndrome and renal oncocytosis (1,9). Owing to their occasional coexistence in a sporadic form of hybrid tumors, as well as within the aforementioned syndromes, they are often considered to be the opposite ends of the same morphological spectrum (9).

Although there are helpful histologic differences between RO and ChRCC, such as the frequent presence of a capsule which tends to be thicker in ChRCC, or the presence of 'raisinoid', binucleated and multinucleated cells usually showing perinuclear halos in ChRCC, these are sometimes not enough to confidently direct us towards a final diagnosis (3,5). Another diagnostic method considered to be helpful is electron microscopy, although it is not suitable for daily practice since it is time consuming and expensive, while not being specific or sensitive enough. The ultrastructural analyses show that ROs have more abundant and larger mitochondria, with long and lamellar cristae, while ChRCCs are characterized by numerous cytoplasmic microvesicles with a tendency to perinuclear localization and more dispersed mitochondria (6). The number of mitochondria in ChRCC depends on the number of microvesicles, the two being inversely proportional (6).

A number of immunohistochemical markers were studied for potential difference in expression between RO and ChRCC. Cytokeratin 7 is said to be typically positive in isolated and scattered cells in RO, whereas it should show diffuse and strong staining in ChRCC, although some studies show that ChRCC can be completely negative and RO can be positive for it (4,10). A number of other markers were studied, among many are vimentin, CD10, CD117, EMA, racemase, MOC31, MAGE-A3/4 and NY-ESO-1, but none of them proved to be consistently positive or negative and thereby they are not acceptable for differentiation (7, 11-14).

Caspases are the key effectors in the execution of apoptosis. They are synthesized as proenzymes, which become proteolytically cleaved during apoptosis to generate active enzymes. Apart from being present in the cytosol, procaspases are also localized in other subcellular compartments such as mitochondria (15). Owing to the high content of mitochondria present in ROs but not ChRCCs, we analyzed the expression of caspase 3 in these tumors. We found that both ROs

and ChRCCs expressed caspase 3, although the ISI was significantly higher in ROs. The ISI was high in 25% of ROs, whereas the majority of ChRCCs had low ISI. We think that the stronger and diffuse staining for caspase 3 in ROs could have resulted from the higher content of mitochondria. A study by Kowalewski *et al*. (16) showed different results than ours; they found that the level of caspase 3 was significantly higher in ChRCCs than in ROs. The difference between the results could be a consequence of utilization of different clones of antibodies and protocols, as well as different scoring systems. Kowalewski *et al*. also analyzed the expression of survivin, which was negative in all cases of ChRCC, whereas only 5% of the ROs were positive (16).

The importance of differentiating RO from ChRCC lies in their different biological behavior and hence different treatment options. Although both of these tumors are most commonly treated by partial or complete nephrectomy, active surveillance could also be applied. According to Richard *et al*. (17), ROs and to some extent ChRCCs, can initially be safely followed with regular imaging techniques, since their annual growth rates are low. Certainly, prior to employment of such management, histologic diagnosis must be obtained.

CONCLUSION

Oncocytoma and ChRCC are tumors with appreciable histologic overlap but different biological behavior. Although many immunohistochemical markers have been studied in these tumors, none has proved to be specific or sensitive enough to differentiate them. The use of caspase 3 could favor the diagnosis of RO in cases where the tumor shows strong and diffuse staining. Further studies on a larger number of cases are needed to confirm our results.

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S A Ž E T A K

VRIJEDNOST KASPAZE 3 U RAZLIKOVANJU ONKOCITOMA OD KROMOFOBNOG KARCINOMA BUBREGA

F. ČAKAR[1] i T. REGOVIĆ DŽOMBETA[1,2]

[1]Sveučilište u Zagrebu, Medicinski fakultet, Zavod za patologiju i [2]Klinički bolnički centar Sestre milosrdnice, Klinički zavod za patologiju i citologiju Ljudevit Jurak, Zagreb, Hrvatska

Cilj: Onkocitom bubrega i kromofobni karcinom bubrega su tumori epitelnog podrijetla s preklapajućom histološkom slikom, što čini njihovo razlikovanje jednim od izazova u uropatologiji. Cilj ovog istraživanja bio je procijeniti izraženost kaspaze 3 u onkocitomu i kromofobnom karcinomu bubrega u svrhu potencijalne primjene tog imunohistokemijskog biljega u njihovu razlikovanju. ***Metode:*** U istraživanju su analizirana 24 histološki potvrđena uzorka onkocitoma i 24 uzorka kromofobnog karcinoma bubrega iz arhiva Kliničkog zavoda za patologiju Ljudevit Jurak, KBC-a Sestre milosrdnice u Zagrebu. Imunohistokemijska analiza provedena je semikvantitativno primjenom indeksa imunohistokemijskog bojanja *(immunohistochemical staining index,* ISI). Za statističku analizu primijenjen je Mann-Whitneyjev U test. ***Rezultati:*** U svim uzorcima bila je prisutna pozitivna imunohistokemijska reakcija na kaspazu 3 pri čemu je većina onkocitoma imala umjeren ISI, a većina kromofobnih karcinoma nizak ISI. ***Zaključak:*** Kaspaza 3 bi mogla naći svoju primjenu u usmjeravanju dijagnoze prema onkocitomu bubrega u slučajevima kada je prisutna jaka i difuzna reakcija tumora na ovaj imunohistokemijski biljeg. Unatoč tome, potrebna su daljnja istraživanja na većem broju uzoraka kako bi se potvrdila vrijednost naših rezultata.

Ključne riječi: kaspaza 3, onkocitom bubrega, kromofobni karcinom bubrega, imunohistokemija