

## ORIGINAL PAPER

# Transitional cell tumors of the ovary: a compact group with a heterogeneous histological and immunophenotypical pattern

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### Abstract

A small percentage of ovarian neoplasms are transitional cell tumors, which proves to be a distinct group with various histological and immunohistochemical patterns. In this study, 13 archived formalin-fixed paraffin-embedded samples of transitional cell tumors of the ovary have been assessed using standard HE stain and the indirect tristrial ABC peroxidase IHC method for 11 antibodies (CA125, CK7, CEA, EMA, MNF116, CK20, Vim, ER, PgR, PCNA, Ki-67). More than 50% were malignant Brenner tumors. CA125 was positive in all malignant tumors (of Brenner type and transitional cell carcinomas), but not in benign and borderline tumors, while CK7 was positive in ~70% of all cases. These two antibodies have shown a high sensitivity and low specificity, but do not correlate to each other. PCNA was positive in the study batch with a mean value of 40% and Ki-67 with a mean value under 25%. A direct correlation statistically significant has been noted between the aforementioned proliferation factors and the tumor grade ( $r = 0.4$ ,  $p = 0.05$ ). The other markers were unspecific, with low sensitivity and independently of the histopathological type.

**Keywords:** Brenner tumors, immunophenotype, statistics and digital imaging analysis.

### ■ Introduction

Transitional cell tumors of the ovary represent ~2% of all ovarian tumors and according to WHO, depending on the histopathological pattern, they are classified as benign, borderline or malign Brenner tumors and transitional cell carcinomas [1].

The data from the literature show that the benign Brenner tumors may arise on an urothelial metaplasia of coelomic epithelium of the ovary [2] or might be a variant of an adenofibroma, consisting of transitional epithelium, associated with abundant fibrous stroma [3]. Sometime the cellular nests contain microcysts or pseudoglandular spaces with a seromucous secretion [4].

The borderline counterparts (proliferative Brenner tumors) have papillary structures with a fibro-vascular core covered by a transitional epithelium and these types express p63 at immunohistochemistry (IHC).

The malignant Brenner tumors are transitional cell carcinomas with benign or borderline Brenner component and at IHC they express CA125, uroplakin and thrombomodulin. All types of Brenner tumors express CK7.

The transitional cell carcinomas of the ovary have no Brenner component and besides CK7 and

CA125 also express mesotelin [5]; the differential diagnosis is made with metastases from a transitional cell carcinoma of the bladder or undifferentiated serous carcinomas of the ovary.

The prognostic of malignant Brenner tumors is relative favorable, compared to transitional cell carcinomas of the ovary, while the borderline Brenner tumors do not metastasize, but have a recidivist potential.

### ■ Material and Methods

#### Tissue samples

We have retrieved randomly, from our database, in an interval of 9 years, 13 archived formalin-fixed paraffin-embedded samples of transitional cell tumors of the ovary.

The mean age of the women from the studied batch was 47 years (SE = ±2.5).

Sections were cut at 5 µm and stained using the standard HE stain and PAS.

#### Immunohistochemistry

The indirect tristrial ABC peroxidase immunohistochemical method was used for a panel of 11 antibodies, shown in Table 1.

**Table 1 – The antibodies used in the study batch**

Antigen	Producer	Clone	Dilution	Specificity
CA125	Novocastra	Ov185:1	1:100	Ovarian surface epithelium
CK7	DAKO	OV-TL 12/30	1:50	Simple epithelia
CK20	Novocastra	PW31	1:100	Simple columnar epithelium
EMA	DAKO	E29	1:50	Epithelium membrane antigen
MNF116	DAKO	MNF116	1:50	Pan cytokeratin
Vimentin	DAKO	V9	1:100	Intermediate filament
ER	Neomarkers	1D5	1:100	Estrogen receptor
PgR	Neomarkers	1A6	1:50	Progesterone receptor
PCNA	DAKO	PC10	1:200	Proliferating cell nuclear antigen
Ki-67	DAKO	MIB1	1:50	Proliferating factor
CEA	DAKO	II-7	1:25	Carcinoembryonic antigen

The immunohistochemistry (IHC) was performed on 3 µm thick sections from 10% formalin fixed paraffin embedded tissues, according to the indirect tristadial Avidin–Biotin–Complex method of Hsu SM *et al.* [6], modified by Bussolati G and Gugliotta P [7].

Briefly, the procedure was: deparaffinization in xylene and alcohol series, rehydration, washing in phosphate saline buffer (PBS), incubation with normal serum, for 20 minutes, incubation with primary antibody overnight, standard labeled streptavidine-antibody biotin (LSAB) kit (DAKO), washing in carbonate buffer and development in 3,3'-DAB hydrochloride/H<sub>2</sub>O<sub>2</sub>; microwave antigen retrieval in M-citrate buffer pH 6.0 was performed for certain antibodies. All specimens were counterstained with Meyer's Hematoxylin, examined and photographed on a Nikon Eclipse E600 microscope. To ensure the reliability of the experimental study, internal quality control of immunohistochemical techniques was performed as a part of an implemented and certified quality assurance system (ISO 9001/2001).

## Statistics

Descriptive statistics was used for uniform distributed data of the study batch and for correlation between parameters, statistical analysis has been done using the Student *t*-test, “paired two samples for mean” variant, one-group two tails, from the Analysis Tool Pak of Microsoft Excel 2003, running under Windows XP Professional. A value of *p*<0.05 was considered significant.

## Results

The classic histopathology investigation has shown one case (7.69%) with benign Brenner tumor (Figure 1), three cases (23.07%) of borderline Brenner tumors (Figure 2), seven cases (53.84%) of malignant Brenner tumors (Figure 3) and two cases (15.38%) of transitional cell carcinomas (Figure 4), one of them having cellular polymorphism and nuclear atypia, with frequent mitoses.

Three cases had some peculiar aspects: one borderline Brenner tumor with embryonic nests of Walthard type, a benign Brenner tumor associated with a mucinous cyst adenoma and a Brenner tumor with metaplastic areas and microcysts containing a PAS positive secretion (Figure 5).

Immunohistochemically, CA125 was positive in transitional cell carcinomas and in malignant Brenner tumors (Figure 6) and was negative in benign and borderline tumors. CK7 was positive in 10 (70.69%) of 13 cases (Figure 7).

CA125 and CK7 have shown a high sensitivity and low specificity. There was no correlation between CA125 and CK7.

CEA was positive in just three cases (23.07%) and vimentin was positive only in four cases (30.07%). MNF116 and EMA were inconsistently positive (Figure 8), both in Brenner tumors and transitional cell carcinomas, having no correlation with the tumor type. CK20, ER and PgR were negative in all cases.

PCNA was positive in the tumor cells nuclei in all studied cases with a mean value of 40% and Ki-67 was positive in majority of cases, with a mean value under 25%. A reasonable correlation statistically significant (*r*=0.4, *p*=0.05) has been noted between the proliferation factors (PCNA and Ki-67) and the tumor grade; therefore, they might be considered as predictive factors for the subsequently evolution of the tumor.

The other markers were unspecific, with low sensitivity and independently of the histopathological type.

## Discussion

The aforementioned histological and immunohistochemical features show that the transitional cell tumors of the ovary are a distinct entity, with a variable IHC pattern, supported by data from the literature.

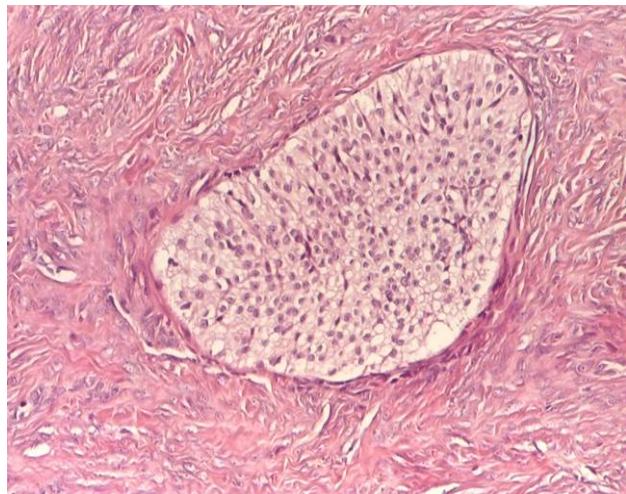
In this context, according to one study, Brenner tumors, but not transitional cell carcinomas of the ovary show urothelial differentiation, with a broadly expression of CK7 and a focally strong expression of CA125 [8].

In addition, in two cases reported by some authors, malignant Brenner tumors of the ovary may show expression of CA125, CA72-4 and surface epithelial cytokeratins, but these antigens should be correlated with serum markers of the same type [9].

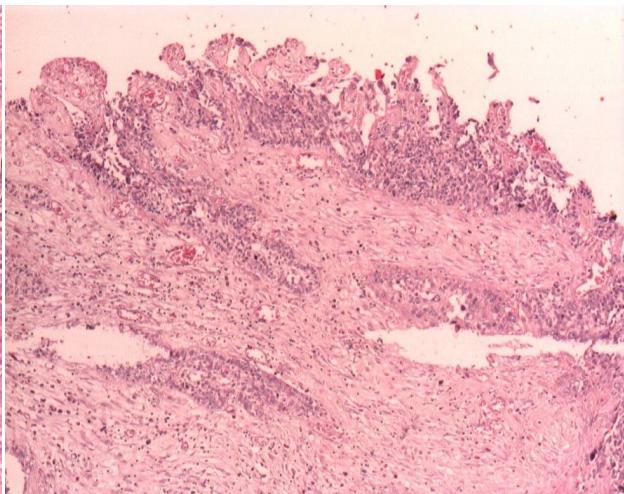
Another study has shown that morphologic similarity between transitional cell carcinoma of the ovary and its counterpart from the urinary bladder does not indicate any histogenic similarity, but CK7, CK20, together with uroplakin III and WT1 may prove useful in distinguishing primitive transitional cell carcinomas of the ovary, and metastases from invasive transitional cell carcinoma of the bladder to the ovary, the previous being a variant morphology in the spectrum of surface epithelial carcinomas [10].

Recent findings have shown that p63 is expressed in benign and borderline Brenner tumors, but not in malignant counterparts and transitional cell carcinomas of the ovary [11], suggesting that this

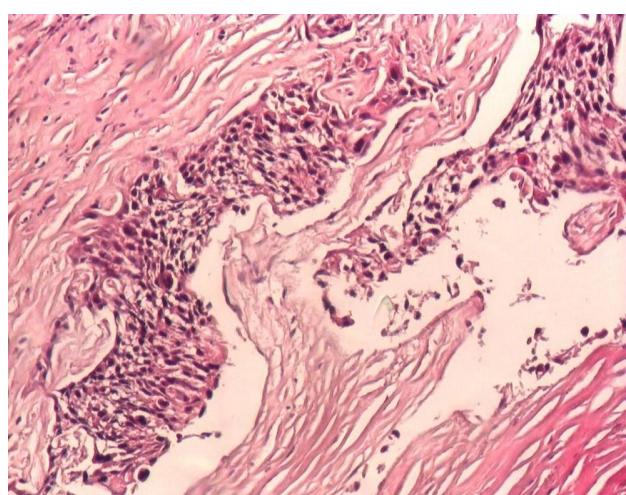
antigen could be a marker for differential diagnosis of malignant Brenner tumors and transitional cell carcinomas, and also playing a role in Brenner carcinogenesis.



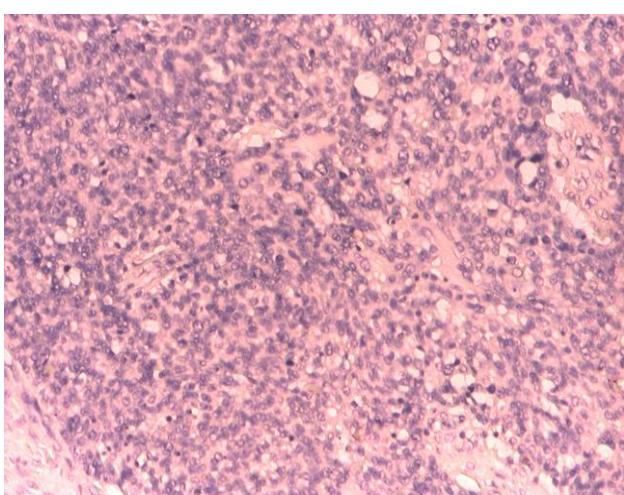
**Figure 1 – Benign Brenner tumor, nest with transitional epithelial cells embedded in a dense fibrous stroma (HE stain, ob. 10×)**



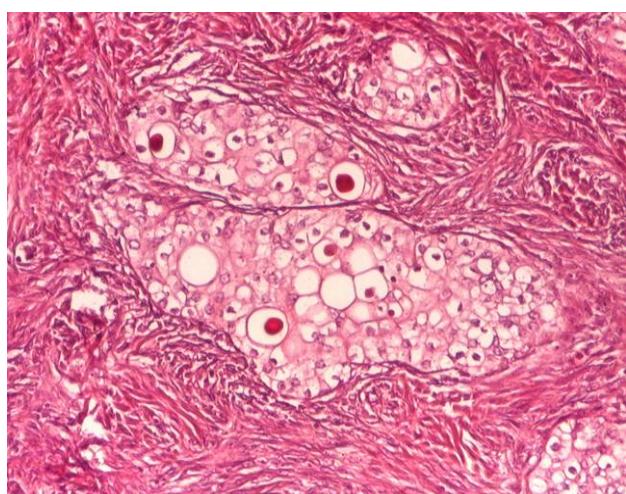
**Figure 2 – Borderline Brenner tumor with epithelial papillae (HE stain, ob. 4×)**



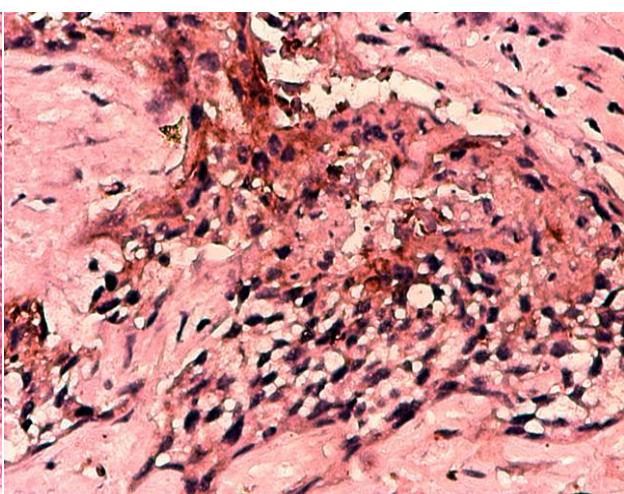
**Figure 3 – Malignant Brenner tumor, cellular polymorphism and atypia (HE stain, ob. 10×)**



**Figure 4 – Transitional cell carcinoma of the ovary, compact sheet with cells showing atypical mitoses (HE stain, ob. 10×)**



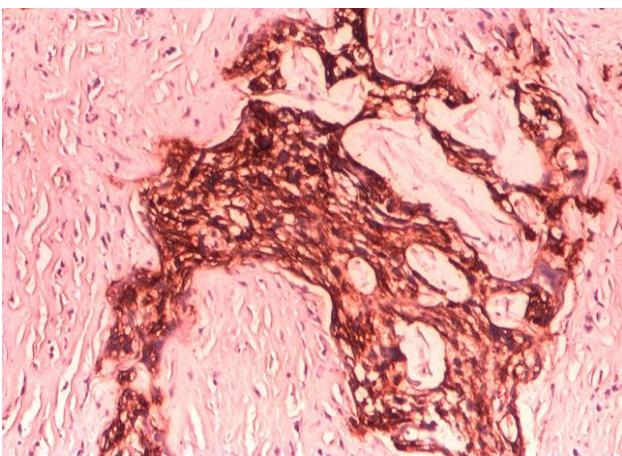
**Figure 5 – Brenner tumor with cystic degenerescence (PAS stain, ob. 10×)**



**Figure 6 – Malignant Brenner tumor, cells staining positive for CA125 (IHC, ob. 10×)**



**Figure 7 – Benign Brenner tumor, diffuse staining for CK7 (IHC, ob. 20×)**



**Figure 8 – Malignant Brenner tumor, positive staining for EMA (IHC, ob. 10×)**

## Conclusions

Although the microscopic examination remains the first tool in the diagnostic of this heterogeneity of tumors, the modern approach should involve besides new IHC markers, also molecular techniques, to separate closely related tumors, to achieve a relevant diagnostic with impact on prognostic and treatment that will better guide patient management.

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Received: April 8<sup>th</sup>, 2008

Accepted: September 25<sup>th</sup>, 2008