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ORIGINAL ARTICLE

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Brenner tumors but not transitional cell carcinomas of the ovary show urothelial differentiation: immunohistochemical staining of urothelial markers, including cytokeratins and uroplakins

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Abstract To determine whether Brenner tumors and transitional cell carcinomas (TCCs) of the ovary are urothelial in type, the immunoprofiles of 14 Brenner tumors, including three malignant examples, and eight ovarian TCCs were compared with those of Walthard nests, urothelium, 12 urinary bladder TCCs and 17 ovarian adenocarcinomas (serous, endometrioid, mucinous, and undifferentiated type). The immunohistochemical stains used included those for cytokeratins CKs 5/6, CK7, CK8, CK13, and CK20, vimentin, CA125, and the specific urothelial differentiation marker uroplakin III. CK7 and CK8 were broadly expressed in most tumors of ovary and bladder examined, while vimentin was focally present in some ovarian TCCs and adenocarcinomas. As in normal and neoplastic bladder urothelium, urothelial markers, including uroplakin III, CK13, and CK20, were detected in the epithelial nests of Brenner tumors. Brenner tumor cells also expressed uroplakins Ia and II. CA125 was observed focally in some Brenner tumors. In contrast, TCCs of the ovary and Walthard nests lacked uroplakins and were essentially negative for CK20 and CK13 but quite strongly expressed CA125. This immunophenotype closely resembled that found in ovarian adenocarcinomas. Thus, it appears that the only true urothelial-type ovarian neoplasm is the Brenner tumor, whereas ovarian TCC most likely represents a poorly differentiated adenocarcinoma with a morphologic transitional cell pattern. These results may explain the controversies as expressed in the recent literature concerning TCC of the ovary and establish its place among the ovarian adenocarcinomas of müllerian type.

Keywords Brenner tumor · Transitional cell carcinoma of ovary · Ovarian adenocarcinomas · Cytokeratins · Uroplakins

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Introduction

Transitional cell lesions of the female genital tract represent a spectrum ranging from Walthard nests to ovarian Brenner tumors and transitional cell carcinomas (TCCs) [1, 4, 8, 31, 32, 40, 41]. The common denominator of the above is their histologic resemblance to urothelium and to its neoplasms. It is generally accepted that transitional cell lesions of the ovary and the fallopian tube arise from transitional cell metaplasia of the ovarian (and fallopian tube) surface epithelium [2, 37, 45].

Notwithstanding the histologic similarities of ovarian TCCs and other transitional cell lesions of the female genital tract to normal urothelium and to carcinomas of the urinary bladder, immunohistochemical [6, 14, 17, 18, 41] and ultrastructural studies [15, 30, 33, 34] have so far been inconclusive in establishing the urothelial nature of these lesions. In electron microscopic studies [15, 30, 34], some similarities with urothelial cells were reported, but those findings cannot be considered as conclusive proof of urothelial differentiation since asymmetric unit membrane (AUM) structures, which constitute the most specific element of urothelium, have not been described in these publications. Furthermore, the study of Quezado et al. [29] has shown that ovarian and bladder TCCs have distinct genetic mutation patterns.

In the present study, a series of Brenner tumors and TCCs of the ovary were investigated with a battery of immunohistochemical stains, including different cytokeratins (CKs), vimentin, CA125 and the recently described uroplakins. The latter are transmembrane proteins constituting the asymmetrical unit membrane of urothelial umbrella cells and are the first specific urothelial differentiation markers described [48, 49, 50]. The results were compared with those obtained in ovarian surface epithelium, in Walthard nests, in a variety of primary ovarian adenocarcinomas, in TCCs of the urinary bladder, and in normal urinary bladder urothelium. The study was intended to establish the immunoprofile of transitional cell lesions of the ovary and their relationship to urinary bladder urothelium and its tumors. The aim was to clarify whether Brenner tumors and TCCs of the ovary express urothelial differentiation in a cell biological sense as opposed to a mere morphological resemblance.

Materials and methods

Paraffin blocks containing formalin-fixed and paraffin-embedded specimens of various normal and malignant human tissues were taken from the archival files of the collaborating Departments of Pathology at Indianapolis, Marburg, and Tel-Aviv. Some tissues were from the Institute of Pathology, Martin Luther University of Halle-Wittenberg, Halle (Saale), Germany.

For the analysis of normal adult urothelium, three surgical specimens from urinary bladder (resected for diverticula) were used, one of which also included a segment of ureter. The lining urothelium of these specimens did not show any pathological changes with light microscopic criteria. In addition, 11 uterine tube specimens containing Walthard nests were also studied.

The tumors used in this study comprised 11 typical benign and three malignant Brenner tumors of the ovary, eight TCCs of the ovary, 11 serous, four endometrioid, one mucinous adenocarcinoma, one undifferentiated carcinoma of the ovary, and 12 TCCs of the urinary bladder. All of these were primary tumors.

For immunohistochemistry, 4-µm thick sections were dried overnight at 58°C. After deparaffinization and rehydration, endogenous peroxidase activity was blocked using 1% H_2O_2 in methanol for 30 min. The slides were then immersed in 10 mM sodium citrate (pH 6.0) and heated in a microwave oven three to five times for 5 min at 600 W [38]. After cooling to room temperature, sections were treated for 15 min with 0.001% trypsin in 0.05 M Tris-HCl buffer (pH 7.8) containing 0.001% $CaCl_2$ at 37°C (for the antibodies against uroplakins, the trypsin step was omitted). After incubation with 10% normal horse serum in phosphate-buffered saline (PBS), the primary antibodies (Table 1) were applied at appropriate dilutions for 1 h at 37°C. Bound antibodies were detected using biotinylated anti-mouse immunoglobulin (Ig)G (Vector, Burlingame, Calif.) or biotinylated anti-rabbit IgG (Vector), respectively, diluted 1:100 in PBS (30 min, room temperature) followed by the avidin-biotin-complex (ABC) peroxidase method (ABC Elite Kit, Vector). The staining reaction was performed using 3,3-diaminobenzidine and H_2O_2. For mild counterstaining, Mayer's hematoxylin solution was used. For negative controls, which always yielded the expected negative results, the primary antibody was replaced with PBS or an irrelevant monoclonal antibody. Photomicrographs were taken using an Olympus AH-3 photomicroscope and Kodak EPY 64T color film or, for black and white pictures, Agfapan 25 film and blue filters.

Results

Adnexal surface mesothelium

The ovarian surface epithelium and the mesothelium covering the fallopian tube were, as all other tissues and tumors studied, analyzed immunohistochemically with monoclonal antibodies to different cytokeratins, vimentin, uroplakin III, and CA125. The mesothelial cells exhibited strong expression of CK7, CK8, and vimentin and heterogeneous expression of CKs 5/6, while CK13 and CK20 were negative. Uroplakin III was totally negative, while CA125 showed heterogeneous immunostaining (not shown). The results are summarized in Table 2.

Walthard nests

Most of the Walthard nests studied were cystic; a few were solid, and one appeared as a surface plaque. They

Table 1 Primary antibodies used

Antibody[a]	Antigen recognized	Source	Reference
D5/16 B4	CKs 5/6	Boehringer Mannheim/Chemicon, Hofheim, Germany	[19]
OV-TL12/30	CK7	Monosan, Uden, The Netherlands	[43]
CAM 5.2	CK8	Becton-Dickinson, Heidelberg, Germany	[21]
2D7	CK13	Monosan, Uden, The Netherlands	[42]
IT-Ks20.8	CK20	Progen, Heidelberg, Germany	[25]
VIM 3B4	Vimentin	Progen, Heidelberg, Germany	[12]
AU1[b]	Uroplakin III	Progen, Heidelberg, Germany	–
Rabbit anti-UP III (A)	Uroplakin III	Own	[26]
Rabbit anti-UP II	Uroplakin II	Own	[26]
Rabbit anti-UP Ia	Uroplakin Ia	Own (Dr. T.-T. Sun)	–
Ov185	CA125	Coulter-Immunotech, Krefeld/Hamburg, Germany	–

[a] All antibodies are monoclonal mouse antibodies except for the rabbit anti-uroplakin antibodies

[b] AU1 is a new monoclonal mouse antibody [immunoglobulin (Ig)G1] raised against bovine asymmetric unit membrane (AUM) material. In Western blotting experiments, it reacts specifically with human uroplakin III. In immunhistochemistry on paraffin-embedded human tissues, it shows specificity for urothelial umbrella cells, identical to the tissue staining profile described previously for the rabbit anti-UP III antibodies [26].The detailed characterization of antibody AU1 will be presented elsewhere (I. Riedel et al., unpublished data)

Table 2 Summary of the immunohistochemical findings for cytokeratins, uroplakin III, vimentin, and CA125 in the different tumor types studied. *TCC* transitional cell carcinomas, *VIM* vimentin

Tissues (n[a])	Score[b] (cytokeratins)	CK8	CK7	CKs 5/6	CK13	CK20	UPIII[c]	VIM[d]	CA125[e]
Brenner tumor of ovary (benign) (*n*=11)	0	0	0	0	0	5[f]	–: 1	–: 5	–: 2
	1+	0	0	4	4	6	+: 10	+: 0	1+: 3
	2+	0	1	2	2	0			2+: 1
	3+	0	1	1	2	0			3+: 0
	4+	7	5	0	3	0			
TCC of ovary (*n*=8)	0	0	0	1	6	6	–: 8	–: 4	–: 0
	1+	0	1	5	2	2	+: 0	+: 4	1+: 1
	2+	3	0	1	0	0			2+: 6
	3+	0	1	1	0	0			3+: 1
	4+	5	4	0	0	0			
Surface epithelial-stromal tumors of ovary (adenocarcinomas)[g] (*n*=17)	0	0	0	11	15	7	–: 17	–: 4	–: 1
	1+	0	0	6	1	10	+: 0	+: 12	1+: 2
	2+	0	0	0	1	0			2+: 6
	3+	0	0	0	0	0			3+: 7
	4+	17	9	0	0	0			
TCC of urinary bladder (*n*=12)	0	0	0	2	1	3	–: 4	–: 7	–: 6
	1+	2	1	5	4	1	+: 8	+: 0	1+: 2
	2+	1	0	4	3	2			2+: 0
	3+	2	1	1	3	1			3+: 0
	4+	7	6	0	1	5			
Adnexal surface mesothelium		+++[h]	+++	++	–	–	–	+++	++
Normal urothelium		+++	++	++[i]	++[j]	++[k]	++[l]	–	–[m]

[a] Number of cases studied. Some antibodies were not tested in the total number of cases; this is evident for each relevant tumor and antibody from the difference between *n* and the sum of the cases given as results

[b] Percent of tumor cells stained for cytokeratins: 0, no staining; 1+, ≤ 10%; 2+, 11–30%; 3+, 31–80%; 4+, 81–100%

[c] Uroplakin III staining of tumor cells: –, no staining; +, positive staining in variable amounts of tumor cells

[d] VIM staining of tumor cells: –, no staining; +, positive staining in variable amounts of tumor cells

[e] CA125 staining of tumor cells: –, no staining; 1+, sparse staining; 2+, moderate staining; 3+, extended staining

[f] The most typical results are underlined

[g] Serous carcinomas, *n*=11; endometrioid carcinomas, *n*=4; mucinous carcinoma, *n*=1; undifferentiated carcinoma, *n*=1

[h] For adnexal surface mesothelium and normal urothelium, the staining intensity was scored as follows: –, negative staining; +, sparse and focal staining; ++, moderate and/or heterogeneous staining; +++, strong and homogeneous staining

[i] CK 5/6 is found in the basal cell layer. The intermediate and superficial cells were weakly stained or negative

[j] CK 13 is expressed only in basal and intermediate cell layers

[k] CK 20 is positive only in the superficial cells, sometimes in upper intermediate cells too

[l] UP III outlines the luminal membranes of the umbrella cells

[m] In one case scarce luminal cells were stained

showed prominent staining not only for simple-epithelial CK7 and CK8 but also for CKs 5/6, the latter often being more pronounced in the basal cell layers (Fig. 1a). In contrast, CK13 and CK20 (Fig. 1b) were mostly negative (except for very sparse positive cells in two and one cases, respectively). Notably, in the Walthard nests, including the luminal cells of cystic nests and of the surface plaque, uroplakin III was absent (Fig. 1c). The Walthard nests were also negative for vimentin. CA125 was detected in all cases with strong, predominantly luminal staining (Fig. 1d).

Brenner tumors

Eleven benign Brenner tumors (Fig. 2a) were analyzed. The simple-epithelial CK7 and CK8 strongly stained the epithelial nests of Brenner tumors. In addition, CKs 5/6 and CK13, which are characteristic for stratified squamous epithelia and urothelium, stained the Brenner tumor nests strongly but in a heterogeneous fashion (Fig. 2b–c). Staining for CK13 was on average more abundant than staining for CKs 5/6; all of these CKs were more pronounced in the peripheral tumor cell layers. In half of the cases, focal staining of mostly luminal cells was obtained for CK20 (Fig. 2d), which is also expressed in urothelial umbrella cells. The CK20-positive Brenner tumor cells mostly were non-mucinous. Most interestingly, uroplakin III, a specific marker for urothelial differentiation, was detected in all but one of the benign Brenner tumors studied. Focal linear immunoreactivity was observed with this antibody along the luminal surface of the epithelium lining some microcysts within Brenner tumor nests and along intracytoplasmic microlumina present in some cells of these nests (Fig. 2e). In some cases, the cells containing uroplakin III-positive intracytoplasmic microlumina exhibited additional cytoplasmic staining with peripheral accentuation. The number of uroplakin III-positive cells was variable and in some cases rather low. Mucinous epithelial cells were

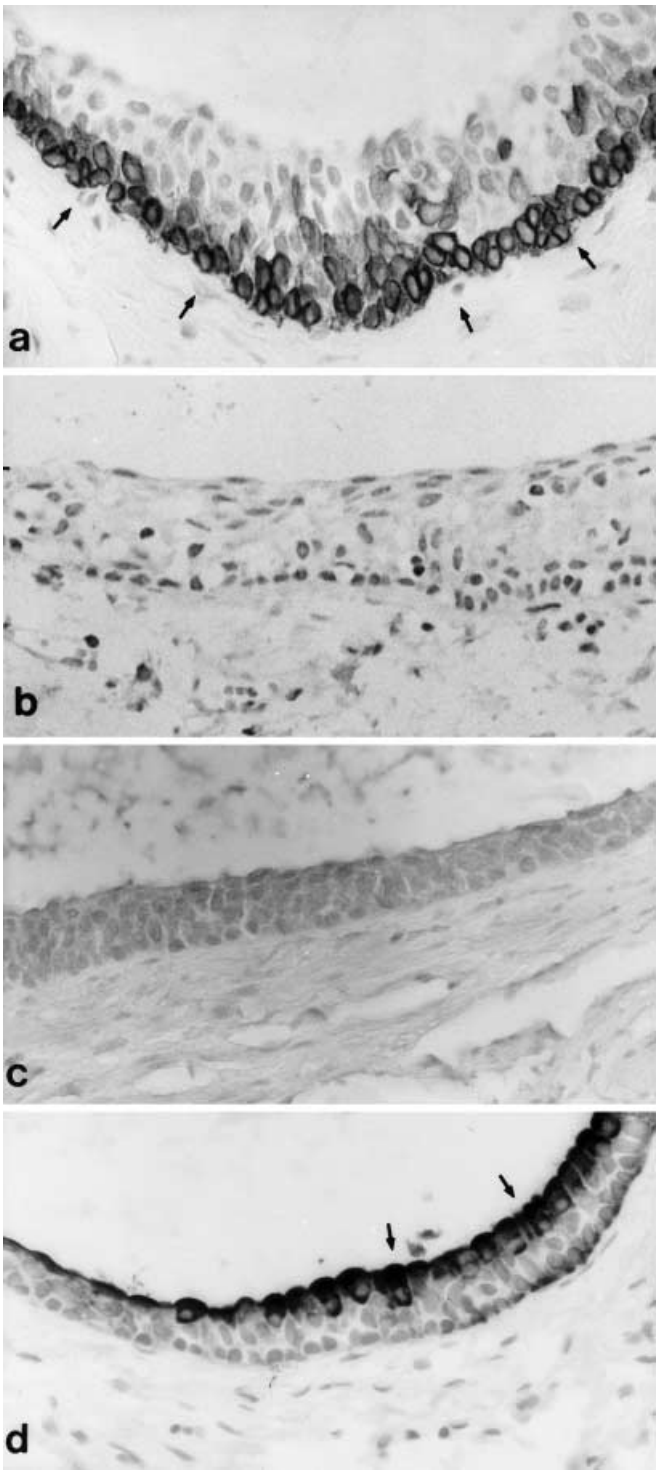

Fig. 1 Immunohistochemical marker pattern of cystic Walthard nests. **a** Prominent staining for CKs 5/6, especially in the basal cell layers (*arrows*). CK20 (b) and uroplakin III **(c)** are negative. **d** Strong expression of CA125 in the luminal cells (*arrows*)

negative for uroplakin III, as were ciliated cells observed in one case. Vimentin was consistently negative in the epithelial nests but strongly stained the stromal cells (Fig. 2f). CA125, a marker for ovarian epithelial tumors, was focally detected in epithelial tumor cells of some but not all Brenner tumors, mainly in the luminal aspect of microcyst-lining epithelium and occasionally within the cytoplasm (not shown).

Four cases of benign Brenner tumors were available for the analysis of two other members of the uroplakin family, i.e., uroplakin Ia and uroplakin II. Using specific rabbit antibodies, both were detected in Brenner tumor nests in a linear pattern at luminal epithelial sites, essentially similar to the distribution of uroplakin III (Fig. 3). On consecutive serial sections, all of the uroplakins analyzed were detected at most sites in identical luminal structures (Fig. 3); however, UPIII seemed to be present in a somewhat lower number of luminal structures as compared with UPIa and UPII.

In the three malignant Brenner tumors studied (data not shown), the benign portions showed marker patterns similar to those of the benign Brenner tumors. In two cases, uroplakin III was detected, and one of these cases showed sparse staining for CK20. In the – morphologically heterogeneous – malignant portions, the immunohistochemical pattern was partly changed. The malignant portion of the first case, histologically resembling intermediate grade papillary TCC with gland-like structures and clear cell areas, showed conspicuously abundant expression of CK20 and an increase in CA125 expression. Interestingly, luminal uroplakin III staining was focally detected in the malignant portion of this Brenner tumor. In the second case, the malignant portion presented as an adenocarcinoma, which showed notable increase of CK20 and CA125 and also co-expressed vimentin, whereas stratified-epithelial CKs were reduced and uroplakin III was absent. The third case, exhibiting close admixture of benign and malignant portions and a proliferating portion resembling non-invasive low-grade papillary TCC with mucinous metaplasia, showed sparse to moderate staining for CA125 and completely lacked both CK20 and uroplakin III.

Transitional cell carcinomas of the ovary

Eight cases of ovarian TCC were studied, three of which were mixed with components of other ovarian epithelial tumors (serous and endometrioid adenocarcinoma). Some tumors were composed primarily of papillary structures lined by transitional cell epithelium. Others also showed a microcystic pattern with some luminal secretion.

The immunohistochemical profile was similar in the various histological patterns encountered. Immunostaining for CK7 (Fig. 4a) and CK8 was generally abundant, with a heterogeneous distribution of CK8 in some cases. CKs 5/6 showed very heterogeneous expression, ranging from negative to staining of about one third of the tumor cells in one of the cases (Fig. 4b). Both CK13 (Fig. 4c) and CK20 (not shown) were present focally in single and/or groups of cells in only two of the cases. Uroplakin III was completely negative (all cases studied; Table 2), and the same was true for uroplakins Ia and II

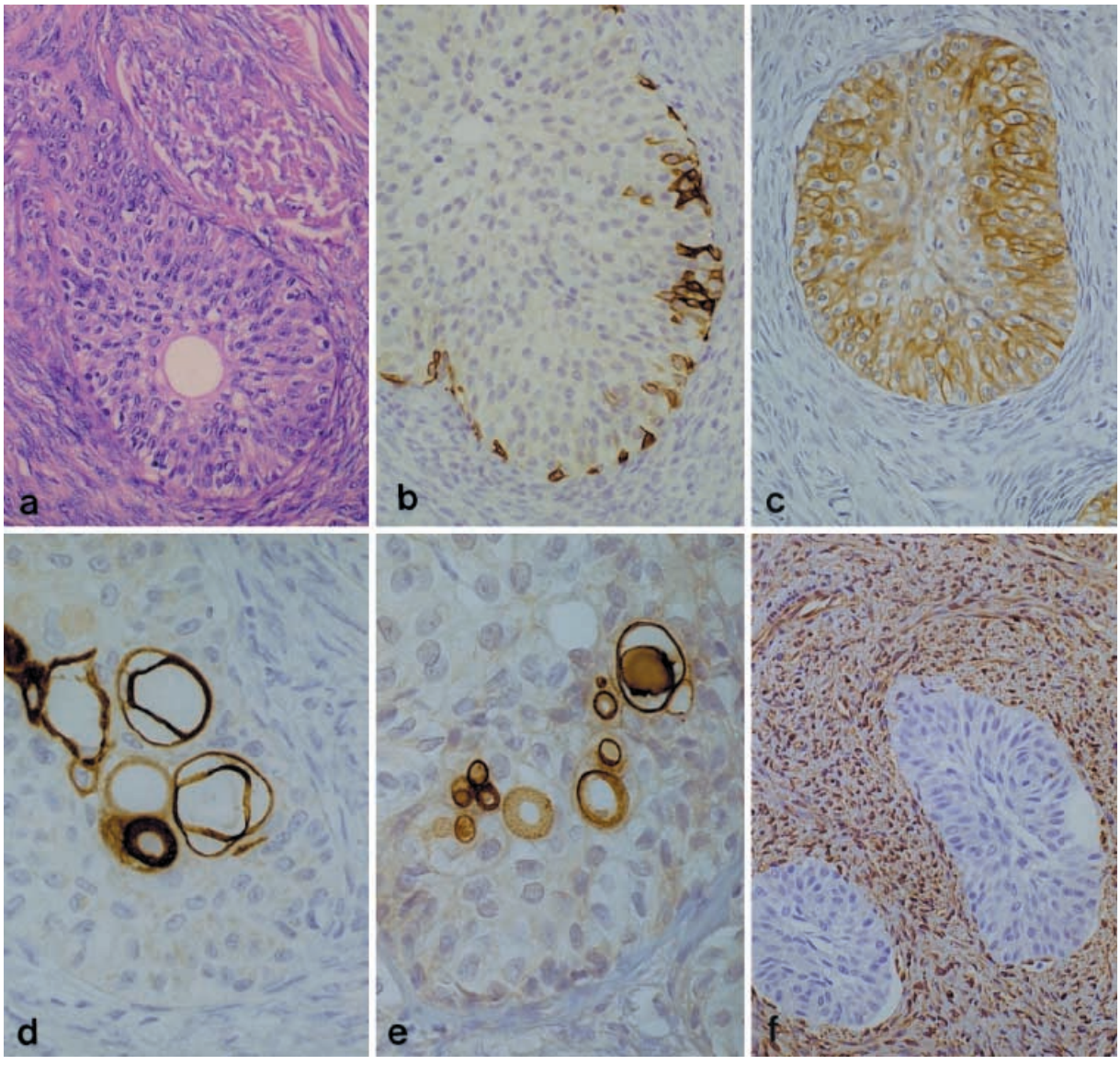

Fig. 2 Immunohistochemical marker profile of benign Brenner tumor. **a** Typical morphological appearance (hematoxylin and eosin). Note the central lumen within an epithelial tumor cell nest. While CKs 5/6 (**b**) and CK13 (**c**) frequently show a peripheral distribution of positive tumor cells, CK20 (**d**) characteristically is expressed in epithelial cells bordering the lumina. **e** Specific linear immunoreactivity along the luminal surface of epithelial Brenner tumor cells for uroplakin III. Note that in some microcysts, the positive membrane is tangentionally sectioned and therefore seems broader. The significance of the staining of the intraluminal secretory material seen in one of the microcysts is uncertain. **f** Negative staining of the epithelial cell nests for vimentin, which is expressed in the stromal cells. **b–f** Immunohistochemical stainings [avidin-biotin complex (ABC) peroxidase method]

(three cases studied; not shown). Four cases showed focal expression of vimentin in carcinoma cells (not shown). CA125 was detected to a considerable extent in most cases of ovarian TCC, especially in the luminal aspects of the tumors and the lining of microcysts (Fig. 4d).

Primary ovarian adenocarcinomas

There were 17 tumors which consisted of 11 serous, four endometrioid, one mucinous, and one undifferentiated carcinoma. Basically, the immunohistochemical results were similar in all of these tumors (not shown). CK7 and CK8 were diffusely present in most tumor cells of all the cases. CKs 5/6 showed minor and focal staining in six cases (four serous, two endometrioid) while CK13 was present in two endometrioid carcinomas only. CK20 was detected at very low levels in five serous and four endometrioid carcinomas and was more pronounced in the mucinous carcinoma. Uroplakin III was consistently negative in all cases. Focal co-expression of vimentin, together with CKs in tumor cells, was seen in eight serous and in all four endometrioid carcinomas and was present in the stromal cells of all the tumors. CA125 was positive in all cases except for the mucinous carcinoma.

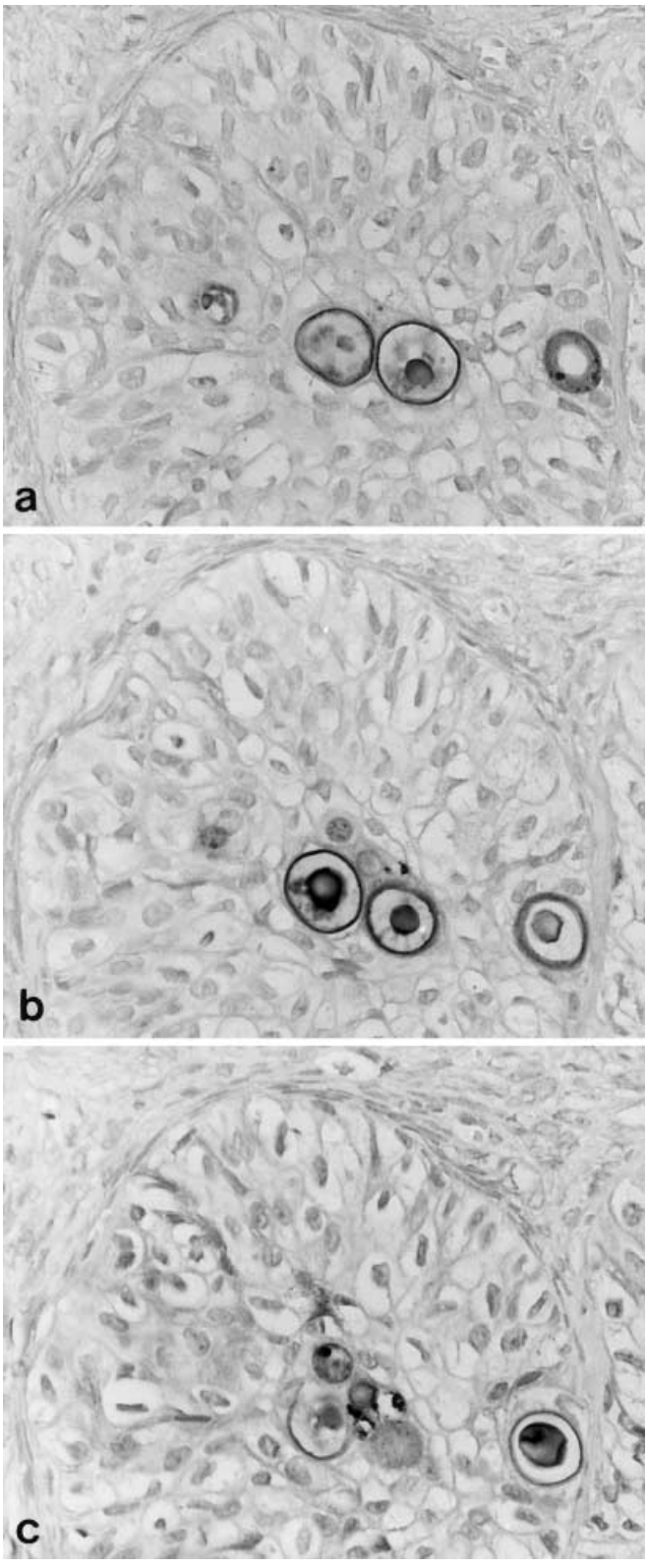

Fig. 3 Comparison of the expression patterns of different uroplakins in benign Brenner tumor as analyzed using immunohistochemical staining of consecutive serial sections: Uroplakin III (**a**), Uroplakin Ia (**b**), and Uroplakin II (**c**). Note that all three uroplakins are present in an epithelial Brenner tumor nest in the same microcystic structures, with linear staining of the luminal cell membranes

Normal urothelium

In the four cases of normal urothelium (three bladders and one ureter; data not shown) there was diffuse immunostaining for CK8 in all layers, while CK7 exhibited heterogeneous patterns and considerable quantitative differences between individual cases. In the ureter, CK7 was homogeneously expressed. CKs 5/6 were found most prominently in the basal layer, diminishing or disappearing in the intermediate layers. CK13 was detected mainly in basal and intermediate cells. In contrast, CK20 was present most typically in superficial umbrella cells and, less frequently, in upper intermediate cells. However, there was conspicuous heterogeneity along the urothelial lining and between individual cases, with the ureter sample staining almost negatively. Uroplakin III was specifically observed in most or all umbrella cells, strongly outlining the luminal membranes. Vimentin was negative in the urothelium. No immunostaining was observed for CA125, except for very scarce luminal cells in one case.

TCCs of the urinary bladder

The 12 cases of TCC of the urinary bladder studied were of grade 2 and grade 3 using the three-grade system. There was invasion of the lamina propria and the muscularis propria in three and nine cases, respectively. CK7 and CK8 were present in all tumors studied, usually being strong and extensive but more limited in a few cases. Focal, usually minor, expression of CKs 5/6 was detected in ten tumors. CK13 was, in general, more pronounced than CKs 5/6 and was seen in 11 tumors (Fig. 5c). Nine of the tumors showed various degrees of CK20 expression, with extensive staining in five (Fig. 5b). Uroplakin III was focally detected in eight tumors and arranged in different patterns, including staining of the membrane lining microlumina, the cytoplasm, and/or the basal plasma membrane of cells at the tumor–stromal interface (Fig. 5a). Vimentin was negative in the tumor cells but positive in stromal cells. CA125 was negative in most of the cases studied but was focally positive in two cases (Fig. 5d).

Discussion

TCC of the ovary has been defined as a carcinoma that presents morphologic urothelial features but, in contrast to malignant Brenner tumor, lacks elements of benign, metaplastic, and/or proliferating Brenner tumor [3]. Since morphologic similarities can be deceptive, attempts have been made in the past to establish whether ovarian TCC (and other transitional cell lesions of the female genital tract; see below) present an immunoprofile, which is also characteristic of urothelial neoplasms of the urinary bladder. Such confirmation would argue for their urothelial differentiation in a cell biological sense.

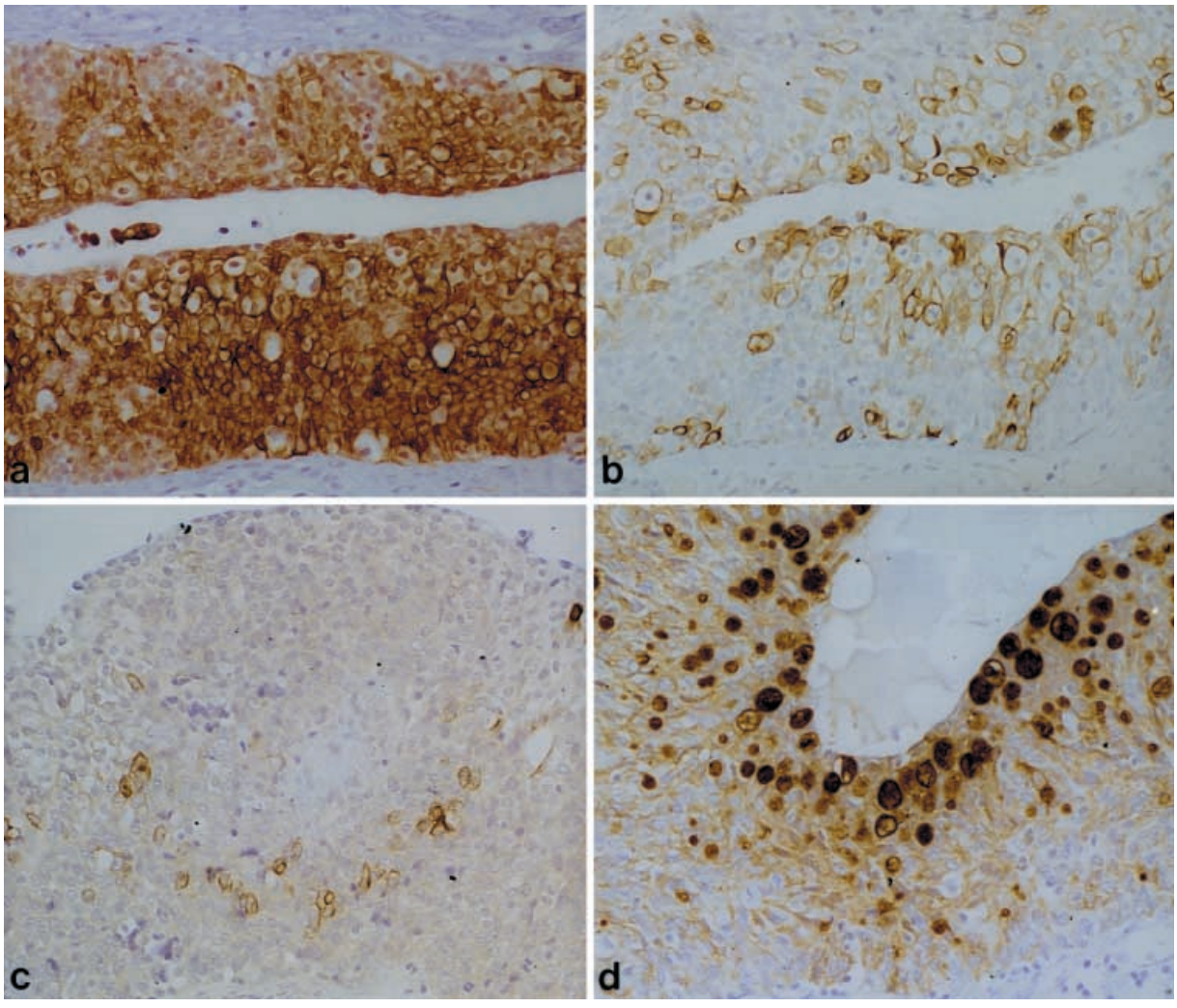

Fig. 4 Immunohistochemical marker pattern of transitional cell carcinoma of the ovary. **a** Abundant immunostaining of carcinoma cells for CK7. **b** Heterogeneous staining for CKs 5/6 without discernible pattern. **c** Very sparse staining of a few tumor cells for CK13. **d** Prominent expression of CA125, mostly in the lining of tiny microlumina

Lifschitz-Mercer et al. [17] demonstrated the presence of CKs of stratified squamous epithelium in the epithelial elements of the Brenner tumor, stressing their potential for squamous differentiation. The staining pattern of bladder urothelium was similar to the latter with the exception of the absence of CK10, characteristic of keratinization.

Soslow et al. [41] used a large battery of antibodies in a study comparing transitional cell proliferations (including Brenner tumors and TCCs) of the ovary with TCCs of the urinary bladder. The most striking result of this study was the essential absence of CK20 in the ovarian lesions relative to those of the urinary bladder. This CK has been demonstrated in several studies to represent a very useful marker for normal and neoplastic urothelium [22, 25]. The above results [41] were confirmed by Kiyokawa et al. [14]. Lininger et al. [18], who studied TCC of the endometrium with CK7 and CK20, also obtained a müllerian immunoprofile (CK20 negative) and not a urothelial one in endometrial TCC.

In contrast to the above-mentioned studies [14, 41], in our own previous study [6], we detected expression of CK20 in most instances of Brenner tumors, suggesting but not proving the urothelial nature of this lesion. The results in ovarian TCC were equivocal, with the poorly differentiated tumors remaining unstained.

Few cases of ovarian and fallopian TCC have been studied by means of electron microscopy [15, 33]. Although some of the ultrastructural features were similar to those described in high-grade TCC of the urinary bladder [46], far reaching conclusions as to the identity of ovarian TCC and bladder TCC cannot be obtained from these studies. Ultrastructural studies have also been performed in Brenner tumors [30, 34], which showed certain resemblance to normal urothelium. However, the presence of the AUM, which is the most characteristic and specific ultrastructural feature of urothelium, was not reported in these tumors.

The above cited publications suggest that, with the possible exception of Brenner tumors, which were essentially negative for CK20 in two studies [14, 41] but

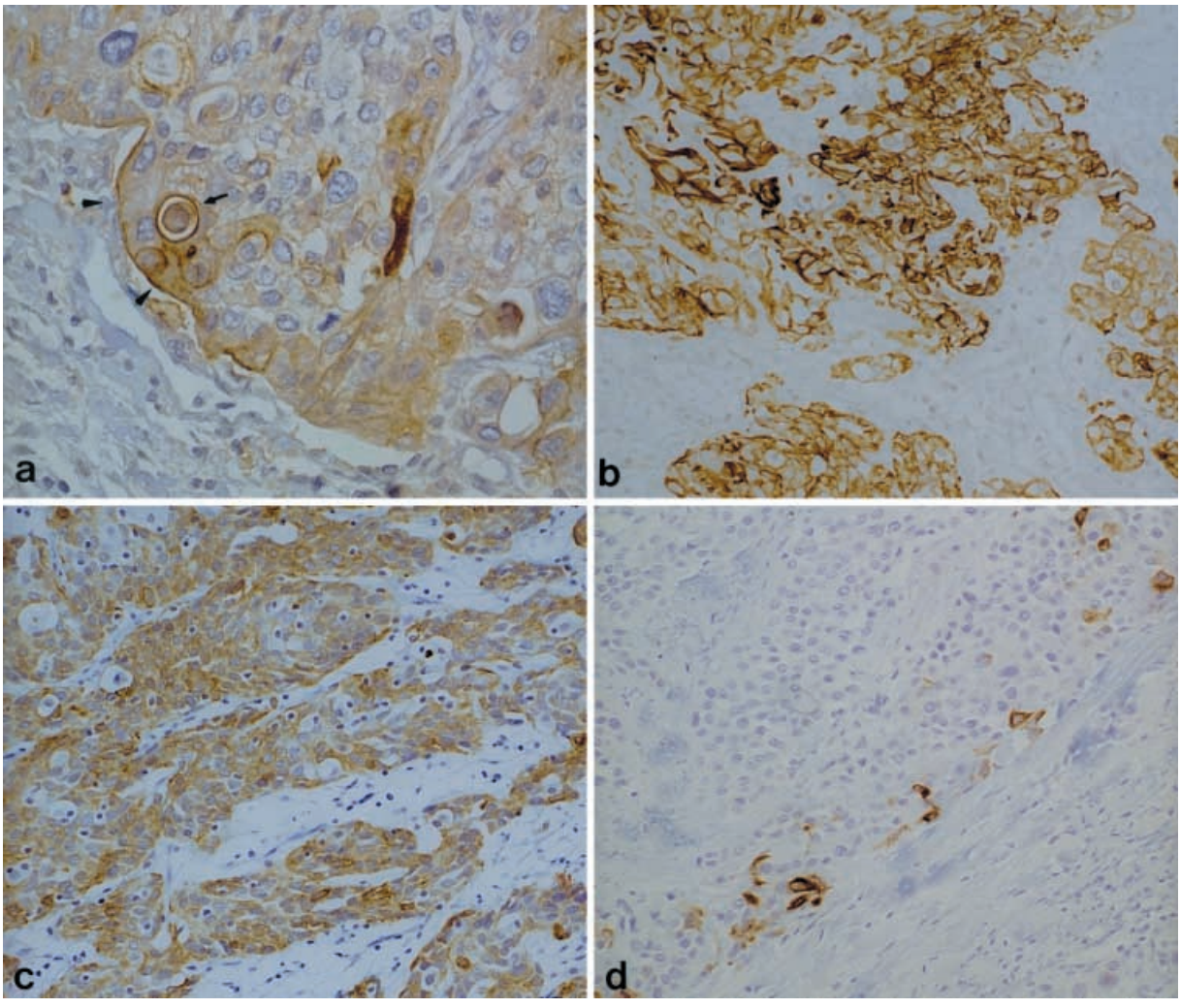

Fig. 5 Immunohistochemical marker pattern of transitional cell carcinomas (TCC) of the urinary bladder. **a** Uroplakin III is seen in the illustrated case not only in the typical luminal pattern (*arrow*) but also in the basal pattern at the tumor–stromal interface (*arrowheads*) and in the cytoplasmic pattern. Examples showing prominent expression of CK20 (**b**) and CK13 (**c**). **d** Exceptional, focal and sparse immunostaining for CA125

stained for this CK in another preliminary study [6], ovarian transitional cell lesions differ in their immunoprofile from urothelium and from tumors of the urinary bladder in spite of morphologic similarities. Using thrombomodulin, another molecule typical of urothelial cells (but also found in several other cell types), a similar difference between these tumor groups has recently been reported [28].

A new and significant aspect of the present study is the application of monoclonal and polyclonal antibodies to uroplakins, which are the first specific urothelial differentiation markers available [26, 48, 50]. The morphologic correlate of these molecules is the AUM, a unique plasma membrane specialization forming numerous plaques that cover the apical surface of urothelial umbrella cells and which are probably involved in strengthening and stabilizing the urothelial cell surface, thus preventing the cells from rupturing during bladder distention (for references, see [48, 50]). The thickened outer leaflet of the AUM is constituted by four major integral membrane glycoproteins, the uroplakins Ia (27 kDa), Ib (28 kDa), II (15 kDa), and III (47 kDa) [48, 49, 50]. Polyclonal [26] and newly raised monoclonal antibodies against uroplakin III (I. Riedel et al., unpublished data) specifically stain the luminal umbrella cell membrane of normal human urothelium. We have previously shown that the majority of invasive TCCs of the urinary tract retain a focal expression of uroplakin III [26, 47]. This has also been confirmed in the present study. In the same previous paper, no uroplakin III staining was found in any of an extended series of non-urothelial carcinomas, supporting the high urothelial specificity of this marker.

Our finding of uroplakin III expression in most cases of Brenner tumors studied can thus be regarded as unequivocal evidence of the true urothelial differentiation proceeding in some cells of these neoplasms. This view is strengthened by the simultaneous detection of uroplakins Ia and II in Brenner tumors. The immunohistochemical pattern revealed by the uroplakin antibodies shows that in Brenner tumors, those tumor cells lining the mi-

crocysts, which are non-mucinous and non-ciliated, correspond at least in part to umbrella cells of normal urinary tract urothelium. These results, therefore, suggest that these microcysts represent some abortive lumen formation analogous to the luminal surface of the lower urinary tract. Recently, using a polyclonal rabbit antiserum against all four uroplakins, Ogawa et al. [27] also reported on focal expression of uroplakins in their five cases of Brenner tumors. In the present study, we confirm and extend these findings, demonstrating the presence of specific uroplakins detected using polyclonal and monoclonal antibodies in a larger series of such tumors.

Further support of true urothelial differentiation in Brenner tumors is also supplied by our finding of the frequent (albeit focal) presence of CK20 in Brenner tumors, confirming our previous preliminary data [6], and the presence of CKs 5/6 and, in particular, CK13 in these tumors. Thus, the CK phenotype of the epithelial component of Brenner tumors is similar to that of normal urothelium and bladder TCCs [23, 25, 35, 36]. The consistent presence of the stratified-epithelial CKs 5/6 is well in line with the known potential of Brenner tumors for squamous differentiation. Also, the expression of these CKs probably is related to the early report of Ganjei et al. [9] on the presence of immunoreactive epidermal prekeratin in Brenner tumors.

As to malignant Brenner tumors, the number of cases available for this study is too small to draw significant conclusions. However, our data show that uroplakins may occasionally be expressed even in malignant parts of Brenner tumors while in other cases, this marker is not expressed at all.

Notably, we found no evidence of urothelial differentiation in the epithelium of Walthard nests. Specifically, the urothelium-typical CK13 and CK20 were mostly negative, while the specific marker uroplakin III was not detected at all. Instead, the ovarian surface epithelium marker CA125 was strongly expressed. We were not able to confirm the finding of Ogawa et al. [27], who noted occasional uroplakin staining of Walthard nests. Thus, according to our results, Brenner tumors and Walthard nests, which were believed to represent related structures [30, 34], appear to be of a different epithelial type. In fact, the immunophenotype of Walthard nests, showing prominent expression of the stratified-epithelial CKs 5/6 but also of CA125, does not conform to any defined normal epithelium, although there appears to be some relationship to mesothelium. Our results do not, however, contradict the accepted histogenesis of Brenner tumors from the surface epithelium of the ovary through a metaplastic mechanism. Indeed, our data are compatible with a true urothelial metaplasia of the surface epithelium, while Walthard nests do not seem to be involved in the development of these tumors.

TCCs of the ovary, by definition, morphologically resemble TCCs of the urinary bladder. Common structural features also include microcysts, which may occur in some urinary bladder TCCs [see 26] but also appear in some ovarian TCCs as small pools filled with mucin [37]. However, in contrast to what we observed in Brenner tumors, our results with TCCs of the ovary showed an immunophenotype that differed considerably from that of TCCs of the bladder. Most notable was the consistent absence of uroplakin III in ovarian TCCs. In addition, CK20 and CK13 were only very rarely expressed in these tumors. CA125, which was absent in all but two bladder TCCs, was found in all cases of ovarian TCCs, often outlining the above-mentioned microcysts (Fig. 4d). Based on the high level of CA125 and the low levels of CK13 and CK20, ovarian TCCs showed an immunohistochemical profile similar to that encountered in primary ovarian adenocarcinomas [5, 20, 24, 25, 44]. Minor, subtle differences between these two tumor types included the lower level of vimentin and the higher level of CKs 5/6 in the ovarian TCCs, the significance of which is not obvious.

Thus, based on the above results, ovarian TCCs most likely should be considered as poorly differentiated surface epithelial-derived müllerian tumors. This conclusion is also supported by the common admixture of ovarian TCCs with serous, endometrioid, undifferentiated, or unclassifiable adenocarcinomas and by the observation that in many instances, the metastases of ovarian TCCs are predominantly serous carcinomas [10, 39]. Furthermore, according to Roth et al. [33], transitions between ovarian TCC and the other types of neoplasms in the “mixed” tumors can be regularly identified. Other microscopic features described in ovarian TCC, such as papillary and fibroepithelial structures, stromal invasion, high mitotic activity, polygonal or spindle epithelial cells, lumina, mucin-producing glands, anaplastic cells, areas of necrosis and vascular invasion [10, 33, 39], and the complexity of expression of intermediate filament proteins (as observed in the present study) are all consistent with poorly differentiated müllerian adenocarcinomas of the ovary (for their intermediate filament profile, (see [24]). The lack of a urothelial immunotype and the absence of staining with uroplakins in these tumors supply significant evidence that these neoplasms are not related to urothelium based on any objective criteria. This is in line with a recent report suggesting that ovarian TCCs have genetic mutation patterns distinct from bladder TCCs [29]. Furthermore, the contention that ovarian TCC has a better response to different chemotherapy regimens than other ovarian carcinomas, suggesting that they truly represent a separate entity [10, 39], has been challenged by Hollingsworth et al. [13], who found no difference in survival or response to chemotherapy in patients with ovarian TCC relative to patients with serous carcinoma.

In a recent study on transitional metaplasia of the uterine cervix, a morphologic entity which is not undisputed [7, 16], Harnden et al. [11] reported the absence of the terminal urothelial markers CK20 and AUM (i.e., uroplakin) in this lesion and, therefore, suggested the use of the term “immature transitional metaplasia”; however, the ability of this lesion to principally undergo urothelial maturation, as would be implicated in this term, remains to be demonstrated. In the light of these findings, also tu-

mors of the uterine cervix, designated as transitional cell neoplasms [1], might lack true urothelial differentiation, although respective investigations are still needed.

In conclusion, using molecular markers, including certain CKs and the recently described uroplakins, we have shown that Brenner tumor cells may embark on a differentiation pathway of urinary tract urothelium. This is, to our knowledge, the first example of true urothelial differentiation that has been observed in derivatives of an organ outside the urinary tract. Notably, there is no obvious relationship between the urothelium and the putative progenitor cells of Brenner tumors (i.e., the müllerian epithelium). Walthard nests which, in the past, have been considered to be closely related to Brenner tumors [30, 34], showed no evidence of urothelial differentiation and, thus, their apparent relationship to Brenner tumors can be questioned. Urothelial differentiation in Brenner tumor cells thus can be regarded as urothelial metaplasia, the occurrence of which underlines the outstanding multipotentiality of the müllerian epithelium.

Ovarian TCC may be regarded as a particularly poorly differentiated form of müllerian adenocarcinoma rather than a tumor undergoing urothelial differentiation. Although retention of the terminology of "ovarian carcinomas with transitional cell pattern" [39] for these poorly differentiated müllerian carcinomas may be justifiable on morphologic grounds, there is no proven inherent relationship between these neoplasms and urothelial carcinoma of the urinary tract with all its clinical, therapeutic, and prognostic implications.

Note added in proof After submission of our manuscript, a paper by O. Kaufmann, J. Volmerig and M. Dietel (Uroplakin III is a highly specific and moderately sensitive immunohistochemical marker for primary and metastatic urothelial carcinomas. Am J Clin Pathol 113:683–687, 2000) appeared, confirming the presence of uroplakin III in Brenner tumors.

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