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Expression of keratin 13 in human epithelial neoplasms

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Summary. The distribution of the 52 kDa keratin 13 was evaluated immunohistochemically, using the AE8 monoclonal antibody. Various squamous and transitional cell epithelial lesions and representative control tissues were studied. This antibody performed adequately in formalin-fixed and paraffin-embedded tissue, but like keratin immunohistochemistry in general, required protease pretreatment. Keratin 13 was found consistently in the suprabasal layers of squamous epithelia of oral cavity, tonsils, larynx, esophagus, lower female genital tract, and transitional urothelium, but it was absent in the epidermis. Generally, various forms of squamous metaplasia were AE8-positive. In dysplasia, AE8 reactivity was considerably decreased or even absent despite the presence of apparent suprabasal maturation. In differentiated squamous cell carcinomas, AE8 immunoreactivity was usually limited to a few cells in the center of the keratinized foci. However, in 10% of squamous cell carcinomas, a significant number of tumor cells was positive. Only well-differentiated urothelial carcinomas showed AE8 immunoreactivity, while poorly differentiated tumors were negative. Interestingly, a Brenner's tumor showed a high number of AE8-positive epithelial cells. Our results show that the expression of keratin 13, as immunohistochemically determined by AE8 antibody, is significantly down-regulated in squamous cell malignancies. Its possible value as an adjunct to diagnosis of dysplasia should be investigated further.

Key words: Keratin – Immunohistochemistry – Squamous epithelium – Transitional cell epithelium

Introduction

Keratins (cytokeratins) are a family of related intermediate filament proteins typically found in epithelial cells. About 30 keratin polypeptides are presently known expressed in a differentiation-dependent manner in various epithelia and in hair. Generally, a simple pattern of low-molecular-weight keratins is found in non-stratified (simple) epithelia and more complex patterns of higher-molecular-weight keratins are typical of stratified, especially squamous cell epithelia (Cooper et al. 1985; Franke et al. 1981; Moll et al. 1982; Sun et al. 1983). Laborious biochemical analysis by two-dimensional gel electrophoresis has been the standard way of determination of the keratin composition of various tissues and tumors (Franke et al. 1981; Moll et al. 1983, 1988; Sun et al. 1985). With the advent of the monoclonal antibodies specific to individual keratin polypeptides, it has become feasible to analyze a large number of tumors for their content of a particular keratin. While immunohistochemical analysis of the keratins has been one of the most successful ways of detection of epithelial origin of tumors (Battifora et al. 1980; Cooper et al. 1985; Huszar et al. 1986; Moll et al. 1988; Osborn and Weber 1983; Weidauer et al. 1986) a few subdivisions of the epithelial tumors by antibody specific to a single polypeptide have also been launched. For example, keratin 18 is expressed in adenocarcinomas, but not in differentiated squamous cell carcinomas (Ramaekers et al. 1983). Keratin 7 is found only in a subset of adenocarcinomas, specifically excluding colorectal carcinomas, and has been suggested to discriminate between upper and lower gastrointestinal tract tumors (Osborn et al. 1986).

Monoclonal antibody AE8, developed by Sun et al. [previously called CA20 (Cooper et al. 1985)], is specific for the keratin number 13 (M_r 52 kDa). Keratin number 13 is normally present in the non-keratinizing internal squamous epithelia of oral cavity, esophagus, larynx, and uterine cervix, and in the transitional epithelium (Achtstätter et al. 1985; Huszar et al. 1986; Moll et al. 1982; Nagle et al. 1985; Ouhayoun et al. 1985; Sun et al. 1985; van Muijen et al. 1986). This study was performed to evaluate the potential of the monoclonal AE8 antibody as a probe for squamous and transitional cell differentiation in tumors.

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Materials and methods

Five-micrometer sections from formalin-fixed and paraffin-embedded tissue were used for the immunostaining. In parallel, in certain cases fresh frozen sections were cut and air dried at room temperature (RT) for 1 h. The sections were then fixed in acetone (10 min, RT) and stored frozen until immunostaining.

Culture supernatant of the antibody AE8 was used in a dilution of 1:5 to 1:10. Before the immunostaining, sections from formaldehyde-fixed tissue were subjected to a mild proteolytic treatment, as found to be useful for keratin immunostaining in general (Battifora and Kopinski 1986; Miettinen 1989). We used a crude preparation of pepsin (Merck, Darmstadt, FRG) in a concentration of 50 mg/100 ml (0.05%) in HCl, pH 1.8–2.0. The digestion time was 30 min at 37° C. Our experiments showed that such proteolytic treatment greatly enhanced the immunoreactivity for AE8 antibody in the formalin-fixed tissue. The immunostaining was performed with the biotin-avidin-peroxidase method. The primary antibody was preceded by normal horse serum (1:25). The primary antibody was incubated for 1 h, sequentially followed by biotinylated goat antimouse antiserum (Vector Laboratories, Burlingame, Calif.) and avidin combined in vitro with biotinylated horseradish peroxidase. The color was developed with diaminobenzidine supplemented with 0.02% hydrogen peroxidase for 5 min. The sections were counterstained with Mayer's hematoxylin. The immunostainings were assessed semiquantitatively as follows: 0 (negative), 1 (less than 10% positive tumor cells), 2 (10–50% positive tumor cells), 3 (more than 50% tumor cells positive).

Results

In normal squamous epithelia suprabasal cells in the epithelia of oral mucosa, tonsils, larynx, esophagus and uterine cervix were consistently AE8-positive (Fig. 1). The basal cell layer was negative. Epidermis and skin adnexa were negative. These results, initially observed in acetone-fixed frozen sections, were consistently reproduced in paraffin sections of formaldehyde-fixed material. All results are summarized in Table 1.

In normal transitional epithelium basal and intermediate layers of urothelium were AE8-positive. Umbrella cells did not stain with AE8 antibody.

Ninety percent of cases of squamous metaplasia of uterine cervix and larynx, except for the basal cell layer, were AE8-positive (Fig. 2). Whenever there was basal cell hyperplasia, the AE8-negative basal cell zone was widened at the expense of the suprabasal, differentiated, AE8-positive cell layers (Fig. 3). Likewise, islands of squamous metaplasia of the prostate glands often showed AE8-positive cells. A thyroid gland with follicular squamous metaplasia (one case, frozen sections) was negative.

In the dysplastic epithelia of the larynx showed variable staining with AE8 antibody. Fifty percent of cases showed AE8 positivity in dysplastic suprabasal cells, which showed marked decrease in keratin 13 expression as compared to squamous metaplasia (Fig. 2). Case by case comparison is shown in Table 2. In half of the cases, dysplasia showed no reactivity with AE8 antibody, despite morphologically obvious squamous cell differentiation (Fig. 4). The possibility of a masking effect, hiding the epitopes to which antibodies react, has been recognized previously (Franke et al. 1983; Woodcock-Mithell

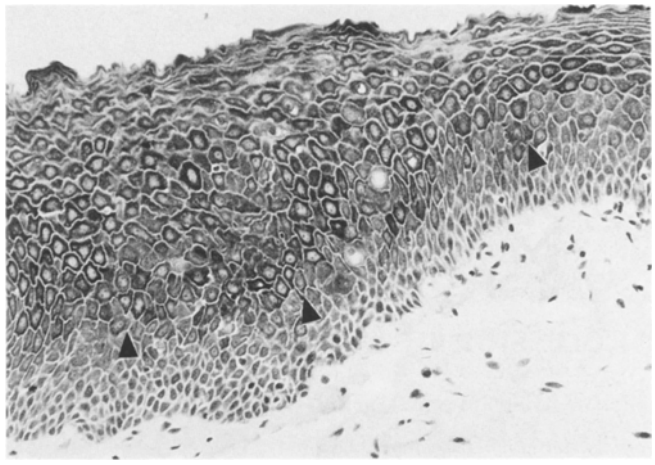

Fig. 1. Normal squamous epithelium of tonsil shows AE8-positive suprabasal cell layer. Hematoxylin counterstain, ×200

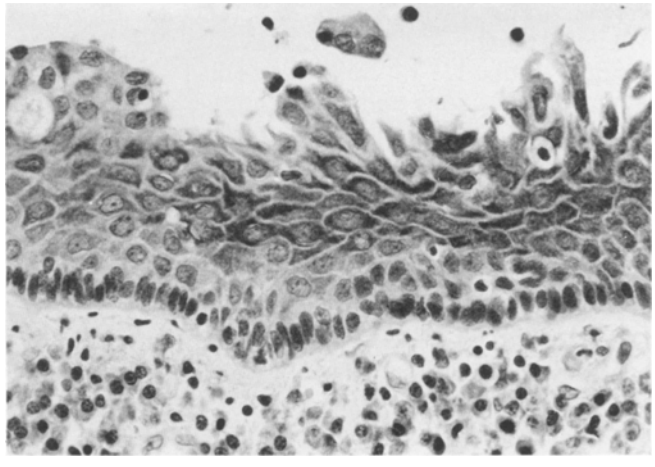

Fig. 2. Squamous metaplasia of the larynx (*right*). The suprabasal cell layer is strongly AE8 positive, while the dysplastic area (*left*) is negative. Hematoxylin counterstain, ×400

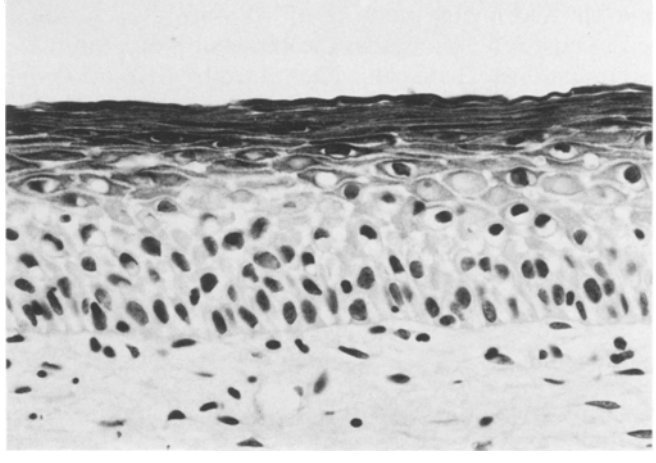

Fig. 3. Squamous metaplasia of uterine cervix with basal cell hyperplasia shows a wide, AE8-negative basal cell zone and positive suprabasal cells. Hematoxylin counterstain, ×400

Table 1. Immunoreactivity of tumors with the AE8 antibody to keratin 13 (combined results of paraffin and frozen sections)

Tumor	NEG	POS(+1)	POS(+2)	POS(+3)	Total
TCC grade I–II	1	0	1	3	5
TCC grade III	8	2	0	1	11
Cervix – squamous ca	1	1	1	0	3
Esophagus – squamous ca	2	4	0	0	6
Larynx – squamous ca	8	4	6	2	20
Epiglottis and pyriform Sinus – squamous ca	1	2	1	0	4
Tongue – squamous ca	1	2	1	1	5
Vulva – squamous ca	1	1	0	0	2
Lung squamous ca	2	2	2	2	8
Lung – adeno ca	7	0	0	0	7
Lung – small cell ca	1	1	0	0	2
Endometr. adenosquamous ca	3	0	0	0	3
Ovary serous papillary ca	8	0	0	0	8
Pancreas adenosquamous ca	1	0	0	0	1
Pancreas – adeno ca	1	1	0	0	2
Nose adeno ca, papillary	1	0	0	0	1
Breast – infiltr. ductal ca	12	0	0	0	12
Kidney – adeno ca	7	0	0	0	7
Prostate – adeno ca	2	0	0	0	2
Ameloblastoma	0	2	0	0	2
Mesothelioma	4	0	0	0	4
Total					
Squamous ca	15	15	10	5	45
TCC	9	2	1	4	16
Adenosquamous ca	4	0	0	0	4
Adeno ca	38	1	0	0	39
Ameloblastoma	0	2	0	0	2
Mesothelioma	4	0	0	0	4
Small cell ca	1	1	0	0	2

NEG, all tumor cells negative; POS(+1), less then 10% tumor cells positive; POS(+2), 10%–50% tumor cells positive; POS(+3), more then 50% tumor cells positive; TCC, transitional cell carcinoma

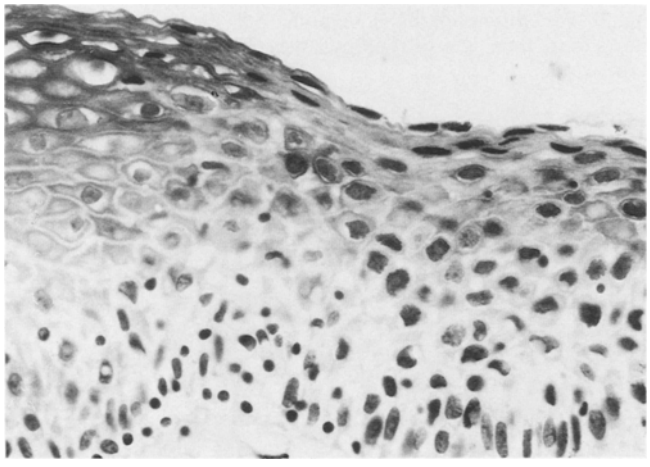

Fig. 4. Cervical mild dysplasia (*right*) is AE8 negative, while the non-dysplastic epithelium (*left*) is positive. Hematoxylin counterstain, ×400

et al. 1982). To address that problem, we immunostained several slides of frozen section material under the conditions described by Franke et al. (1983). These experiments showed no increased reactivity in the tumors examined, suggesting that such epitope masking is not responsible for the limited keratin 13 distribution in squamous cell carcinomas (data not shown). The fact that frozen sections show a similarly restricted keratin 13 pattern indicates that the limited distribution is not a result of lower detection sensitivity in formalin-fixed tissue.

All squamous cell carcinomas of the skin were AE8-negative. Squamous cell carcinomas of the oral mucosa (including tongue and tonsil), larynx, esophagus, bronchus, and uterine cervix showed generally similar patterns of AE8 antibody staining. AE8 immunoreactivity was seen in the cells forming keratinizing nests in "keratinization centers" (Figs. 5, 6). Sheets of tumor cells, despite their well-differentiated suprabasal squamous cell appearance, were mostly negative. However, in about 10% of squamous cell carcinomas, a more extensive distribution of AE8 immunoreactivity was found. In such instances, up to 50% of the total tumor cell population showed AE8 immunostaining (Fig. 7). Fifty percent of well-differentiated squamous cell carcinomas showed no AE8 immunoreactivity (Fig. 8). Poorly differentiated squamous cell carcinomas often lacked the AE8 reactivity entirely. Adenosquamous carcinomas of the endometrium and pancreas were AE8-negative.

Table 2. Pattern of staining of carcinomas, dysplasia and squamous metaplasia of larynx, pyriform sinus, and epiglottis

	Carcinoma	Dysplasia	Metaplasia
1.	1	1	0
2.	0	0	1
3.	2	–	1
4.	1	–	3
5.	2	1	3
6.	0	–	2
7.	2	1	2
8.	2	1	2
9.	2	–	3
10.	3	0	–
11.	0	0	2
12.	2	2	2
13.	0	–	2
14.	0	0	–
15.	0	–	–
16.	1	1	2
17.	2	–	2
18.	1	–	–
19.	0	–	–
20.	0	–	–
21.	–	–	2
22.	0	–	1

All cases were moderately differentiated squamous cell carcinomas of larynx, epiglottis and pyriform sinus
–, Not present in the sections; 0, all cells negative; 1, less than 10% cells positive; 2, 10–50% cells positive; 3, more then 50% cells positive

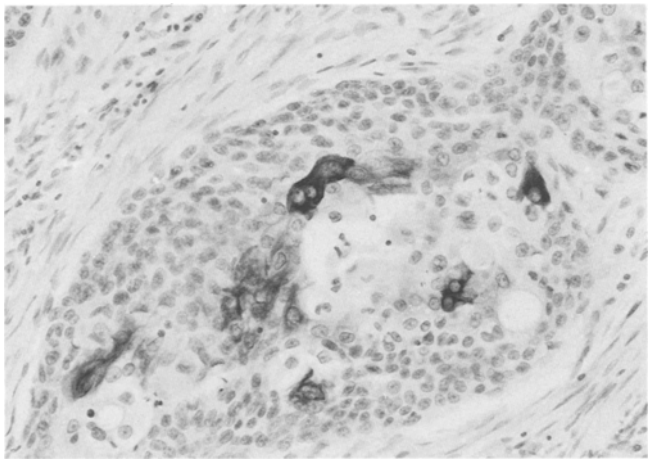

Fig. 5. Squamous cell carcinoma of larynx shows scattered AE8-positive cells localized around "keratinization center". Hematoxylin counterstain, ×200

Among tumors of urothelial origin, only well or moderately differentiated tumors (grade 1 and 2) showed AE8 immunostaining. Poorly differentiated transitional cell carcinomas were notably negative. One case of Brenner's tumor had significant AE8 reactivity in the epithelial cell islands (Fig. 9).

All of the adenocarcinomas (serous papillary carcinomas of ovary, adenocarcinomas of lung, ductal carcinomas of breast, colonic adenocarcinomas) and mesotheliomas examined were consistently AE8 negative. One

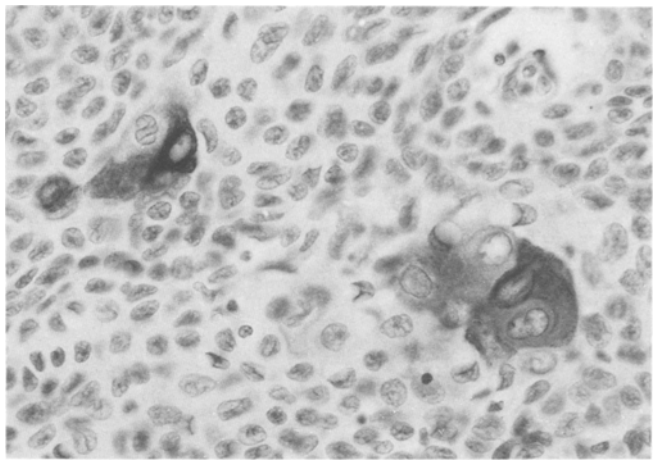

Fig. 6. Higher magnification of squamous cell carcinoma of larynx from Fig. 5 shows scattered AE8-positive cells localized around "keratinization center". Hematoxylin counterstain, ×400

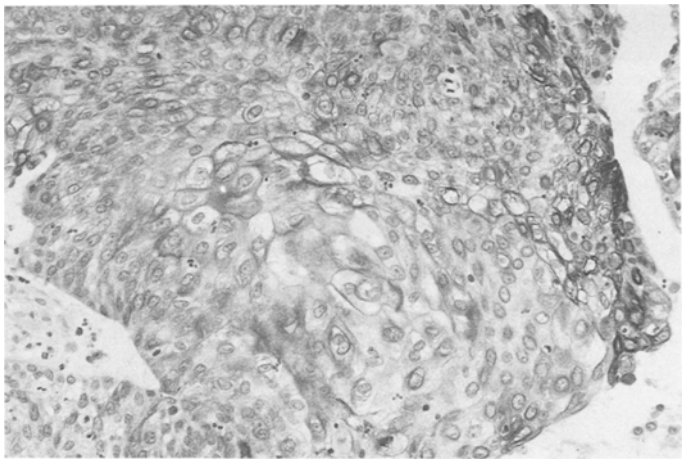

Fig. 7. Well-differentiated squamous cell carcinoma of larynx shows more then 50% of AE8-positive cells. Hematoxylin counterstain, ×400

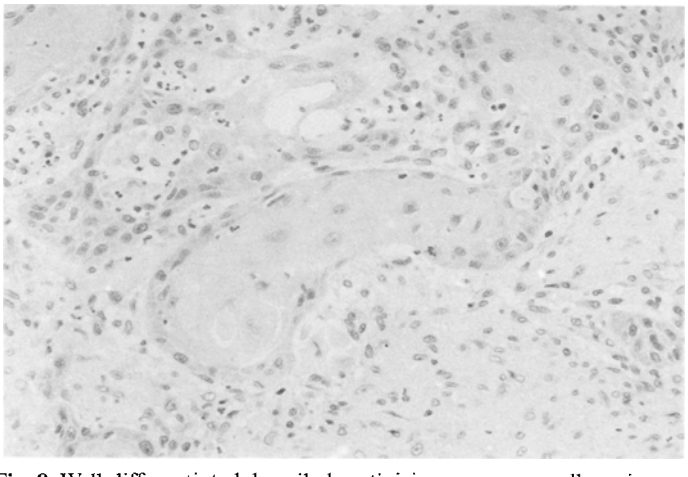

Fig. 8. Well-differentiated, heavily keratinizing squamous cell carcinoma of larynx shows no AE8 immunostaining. Hematoxylin counterstain, ×400

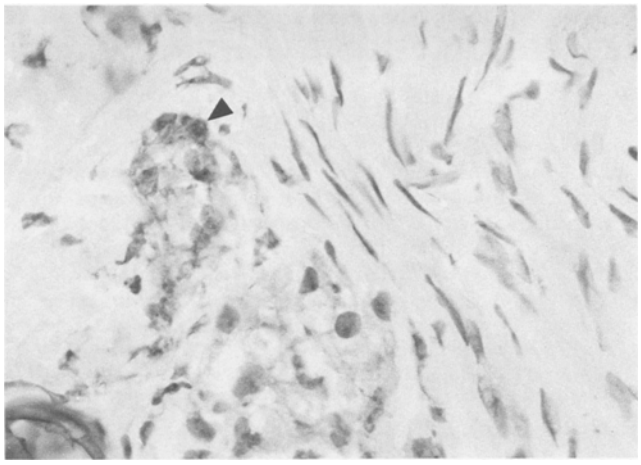

Fig. 9. The periphery of the epithelial nests of Brenner's tumor shows AE8 immunostaining. A focus of calcification is seen in left lower corner. Frozen section; hematoxylin counterstain, × 500

case of pancreatic adenocarcinoma showed scattered, single AE8-positive cells.

Discussion

In this study we have evaluated the distribution of the keratin number 13 (Moll's catalog, molecular weight 52 kDa), by using specific AE8 monoclonal antibody. Our immunohistochemical findings confirmed that this is one of the major keratins in suprabasal cells of the internal stratified squamous epithelia. In many squamous cell carcinomas which appeared to be histologically well differentiated there was, limited keratin 13 expression immunohistochemically. This suggests that keratin 13 expression is down-regulated in squamous cell carcinomas.

Keratin 13 is not present in simple (non-stratified) epithelia. Internal, non-stratified epithelia can acquire keratin 13 only when they are stimulated to undergo stratified differentiation (Huang et al. 1989; Jetten et al. 1989; Nagle et al. 1985; O'Guin et al. 1987). This differentiation process is not irreversible (Jetten et al. 1989; Sun 1989; Tseng et al. 1984); for example, keratin 13 is lost when epithelium cornifies [see Sun (1989) for discussion of keratinocyte differentiation, and for further references].

Squamous cell carcinomas seem to fall into two categories: those that show keratin 13 focally in some cells and those that are devoid of keratin-13-positive cells. Most squamous cell carcinomas of internal squamous epithelia retain some keratin-13-positive cells. The AE8-positive, well-differentiated surface-like cells are concentrated mainly around "keratinization centers". These cells most likely represent the malignant counterpart to the suprabasal and superficial layers of the normal mature internal stratified epithelia.

Interestingly, about 10% of AE8-positive squamous cell carcinomas show more widespread keratin 13 distribution. A majority of the cells in the "keratinization centers" and in the sheets of less differentiated tumor cells were AE8-positive. Extensive AE8-positivity in these cases may represent more prevalent mature suprabasal differentiation of the tumor cells. Whether the extent of the presence of the keratin 13 in moderately/poorly differentiated carcinomas has clinical implications has to be explored by follow-up studies.

Laryngeal squamous cell carcinoma deserves a special note. Squamous carcinomas of this organ are viewed as a whole organ disease by some authors (Resta et al. 1985), implying the existence of various grades of premalignant changes concurrently with carcinomas. In our laryngeal carcinoma cases, we also found areas of dysplasia and squamous metaplasia at a distance from the original tumor. Metaplastic epithelium showed AE8 positivity in the upper suprabasal layers. Intriguingly, the AE8-negative columnar epithelium of larynx can give rise to keratin-13-positive squamous metaplasia and subsequently to keratin-13-positive squamous carcinoma. AE8 positivity in dysplastic epithelium was variable and usually markedly decreased when compared with squamous metaplasia.

Low-grade urothelial transitional cell carcinomas showed moderate keratin 13 immunoreactivity. In contrast, high grade tumors were almost all negative. This suggests significant loss of keratin 13 expression in malignant urothelial tissues, in line with the observations of Moll et al. (1988). The complex nature of the keratin 13 expression in the transitional cell epithelium and transitional cell tumors has been discussed in detail by Moll et al. 1988. The Brenner's tumor in our study showed AE8 reactivity in the epithelial nests, and this is in accordance with the proposed urothelial differentiation of the epithelial component of this tumor (Lifschitz-Mercer et al. 1988).

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