Histol Histopath (1994) 9: 287-294

Histology and Histopathology

Transitional papillary cell carcinoma of the ureter: a histological and ultrastructural study

M. Melis[1], V. Petrozza[1], S. Pulvirenti[1], E. Eleuteri[2], A. Mosca[1], L. Angelini[2] and F. Carpino[1]
[1]Department of Experimental Medicine and [2]Institute of Surgery (IV), University of Rome «La Sapienza», Rome, Italy

Summary. We report a case of transitional papillary carcinoma of the distal left ureter.

The histological and ultrastructural features were seen and compared by SEM and TEM. Observations confirm the data of literature that the deep cells of neoplasm are a normal morphological finding while the superficial cells do not have the characteristics present in normal transitional cells.

In addition, SEM shows small groups or single cells with «pleomorphic microvilli» on the cell surface. These, in ureter, confirm the studies of many authors who have observed these abnormalities in carcinomas of several organs like breast, colon, liver and mesothelium.

Key words: Urothelium, Transitional carcinoma, SEM, TEM

Introduction

In recent years the incidence of transitional cell carcinoma of the ureter (TCC) has increased. This tumour, although rare, accounts for approximately 5% of all primary urothelial tumours (Batata et al., 1975; Whitmore, 1978; Steffens and Nagel, 1988; Das et al., 1990). It is likely that the increase may be partially explained by the more precise gathering and coding of public health data, as well as improvements in diagnostic techniques. TCC is found most frequently in the sixth to eight decades and is twice as frequent in men as in women.

A majority of tumours, 70%, occurs in the lower third of the ureter (Cameron, 1969), while the observations on their preference for either side was not statistically significant (right:left=1:1.2) (Abeshouse, 1956; Grabstald et al., 1971; Highman, 1986; Steffens and Nagel, 1988; Das et al., 1990).

Primary TCC (30-40%) were multicentric, with simultaneous carcinoma in the renal pelvis (32%), in the bladder (2%) and at other sites in the ureter (6%). Synchronous bilateral TCC was rare.

The risk factors for TCC are the following: inflammation and/or calculi, use of tobacco, occupational exposure in certain industries (rubber, painting or paint manufacture, dyestuff, petrochemical or plastic, gas) (Poole-Wilson, 1960; Schade and Swinney, 1968; Sharma et al., 1970; Mahadevia et al., 1983; Steffens and Nagel, 1988) and use of analgesic drugs (aspirin and especially phenacetin) (Poole-Wilson, 1960; Bengtsson, 1962; Buch et al., 1966; Bengtsson et al., 1973; Bennington and Beckwith, 1975; Steffens and Nagel, 1988; Jensen et al., 1989). A high incidence of TCC has been reported in a region of Denmark where abuse of phenacetin is present (Huidt and Feld-Rasmussen, 1973; Jensen et al., 1989) and in regions of Bulgaria, Rumania and Yugoslavia where a high percentage of individuals are afflicted with endemic «Balkan nephropathy» (Petkovic, 1975, 1981).

The majority of transitional cell carcinoma displayed a papillary appearance. According to a majority of authors, these tumours were graded histologically as follows: Grade I (well differentiated); Grade II (moderately well differentiated); Grade III (poorly differentiated).

Furthermore, the tumours were staged (Batata et al., 1975; Batata and Grabstadl, 1976; Das et al., 1990) as follows: Stage A (submucosal infiltration); Stage B (muscular invasion); Stage C (infiltration through the periureteral fat); Stage D (extension outside the ureter involving adjacent structures and/or metastases).

The survival of patients was strictly related to the grade and stage of the tumour. In fact, there was a statistically significative difference of survival between grade I and II, as compared with grade III or IV (Das et al., 1990).

Materials and methods

Case report

A 65-year-old man who was married, a state employee and in apparent good health was presented to

Offprint requests to: Prof. M. Melis, University of Rome «La Sapienza», Department of Experimental Medicine, V. le Regina Elena n. 324, 00161 Rome, Italy

our attention in July 1990, with a history of gross haematuria.

Urographic examination revealed a filling defect of the left ureter in the proximity of its opening into the bladder, leading us to make a diagnosis of ureteral neoformation.

At that time, the patient refused to undergo surgery. Then at the beginning of September 1991, he was admitted to the IV Clinic of General Surgery - Policlinico Umberto I - Rome, after many episodes of gross haematuria. With the exception of the present symptomatology attributed to the urinary apparatus, the patient reported no other pertinent past pathologies. On general examination the patient appeared in good condition. The kidneys were not palpable, the higher and medium ureteric points were not painful and Giordano's maneuver was bilaterally negative.

Another urographic examination was performed before surgery which confirmed the previously described neoformation of the left ureter at its opening into the bladder and the appearance of slight pieloureterectasis (Fig. 1a). A cystoscopy showed a pervious urethra, good vesical capacity with a prominent neck and visible ureteral ostia. Some areas were hyperemic and erosions were present.

The bioptic samples were taken precisely from the following areas: a) left perimeatal zone, where histologically a papillomatous neoformation was present without infiltration of the base of implantation; and b) cupula, which showed an integral covering with small superficial ulcers.

Ultrasonography of the kidney revealed a dys-homogeneous echostructure with parenchymal suffering and moderate left pyelocalicectasia, with ureter dilatation of 8 mm in diameter at the ureteropelvic junction. No lithiasis was present. The bladder's echography showed a slight thickening of the wall and an irregular mucous profile, probably due to a mild hypertrophy of the detrusor muscle; there was no evidence of parietal and/or intraluminal changes. At the level of the fourth hepatic segment, a hyperechogenic formation of about 2.9 cm in diameter was evidenced, referable to a hemangioma. Following a xifo-pubic celiotomy, a nephroureterectomy was performed. The peritoneum was opened and a left parietocholic ligament was detached, with mobilization of the descending colon and splenic flexure. After identifying the ureter, it was isolated to the point where it opened into the bladder. The ureteral opening together with the surrounding 3 cm of bladder wall was excised and successively, cystotomy was performed by utilizing separated point sutures with dexon. After incision of Gerota's fascia and aperture of the perirenal adipose tissue, a normal-sized kidney was reached and subsequently removed together with its ureter. The postoperative period was normal and fever was not present. The suture points were removed after nine days and the patient was discharged on the tenth day.

Tissue preparation

Many fragments were collected directly from the neoformation and its base and from the ureter, below and above the same neoformation, for histological examination. In addition, specimens of the renal pelvis, of the higher part of the ureter and of removed vesical mucosa were also taken.

For light microscopy, fragments were fixed in 10% calcic formaldehyde and embedded in paraffin, cut with a microtome and stained with haematoxilin-eosin, Van Gieson trichromic stain and PAS.

For transmission electron microscopy, the fragments were fixed in 2.5% glutaraldehyde solution in 0.1M. cacodylate buffer at pH 7.3.

Post-fixation was carried out in 1.33% osmium tetroxide. The tissues were then dehydrated in increasing concentrations of ethanol and embedded in Epon 812 (Luft, 1961). Ultrathin sections, obtained with an LKB ultratome III, were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and observed under a Zeiss EM 10 electron microscope.

For scanning electron microscopy, the fragments were fixed in the same glutaraldehyde solution as above, dehydrated in acetone, critical-point dried in liquid carbon dioxide, sputter coated with gold-palladium and examined with a Hitachi S 4000.

Results

Gross features

The kidney (Fig. 1b), with dimensions of 11 x 4 cm presented many foetal lobi and a slightly granular surface. The fat of the renal pelvis was moderately increased, the pelvis was dilated and the renal papilla were diminished in height. The ureter, 21 cm in length, was uniformly dilated up to its distal part, where a large vegetant, arborescent and friable white mass with a broad base of implantation was present (2 cm from its opening into the bladder). The mucosa of the removed bladder section displayed a villous appearance.

Histological features

A histological examination of the deep implantation zone of the neoplasm displayed a solid appearance, which was constituted by packed papillary structures which were covered by cells and abutted on each other, thereby producing a pseudotubular aspect.

As a rule, the longitudinal axis of the cells was at a right angle to the axis of the papilla. On the other hand, a few cells showed an irregular arrangement. In the latter, nuclear atypia with remarkable hyperchromatism was frequent (Fig. 2a). The superficial zone of the neo-formation became gradually papillary in appearance. These papillae were well separated (Fig. 2b-c) and their axes were formed by a connective-vascular stroma with congested and ectasic vessels (Fig. 2d). The major part

of the superficial cells were packed irregularly. Two cellular types were identifiable: the first, with a round nucleus containing granular chromatin in its periphery; and the other, with a hyperchromatic nucleus of fusiform shape or bizzarre and scanty cytoplasm with a clear perinuclear halo. Moreover, many atypical mitotic figures were present (Figs. 2c-d, 3a).

The most atypical cells were located predominantly on the superficial part of these papilla or were collected in the interpapillary spaces due to exfoliation (Fig. 3b).

Around the base of implantation, the ureter appeared thickened with irregular intussusceptions (Fig. 3c), and superficial cells with evident dysplastic characteristics (Fig. 3d) and many Brunn nests were present (Fig. 3e). The dysplastic changes were evident in the upper tract of the ureter for about 2.5 cm and in the lower to its intravesical portion. Below the neoformation, many small thickened groups of atypical epithelial cells were observable around the periureteral connective tissue.

In addition, small papillary formations with slight dysplasia were present in the other fragments of the bladder.

Fibrosis in connection with the convoluted tubule, interstitial phlogosis and vascular sclerosis were visible in the renal tissue.

Aspecific chronic inflammation was evidenced in the pelvic calices and in the upper portions of the ureter.

Ultrastructural findings

TEM observations of a longitudinally cut specimen of papillary proliferation showed a basal cellular layer formed by spindle-shaped cells which were attached at a right angle to the basal membrane, surrounding a fibrovascular core of lamina propria. These cells displayed the ultrastructural features of normal transitional cells: the cell surfaces were connected laterally by tight junctions; lateral intercellular spaces were formed by infoldings of lateral cell membranes; intercellular tonofilaments were present; nuclei had one or more deep convolutions and margination of the heterochromatin; and the cytoplasm had normal

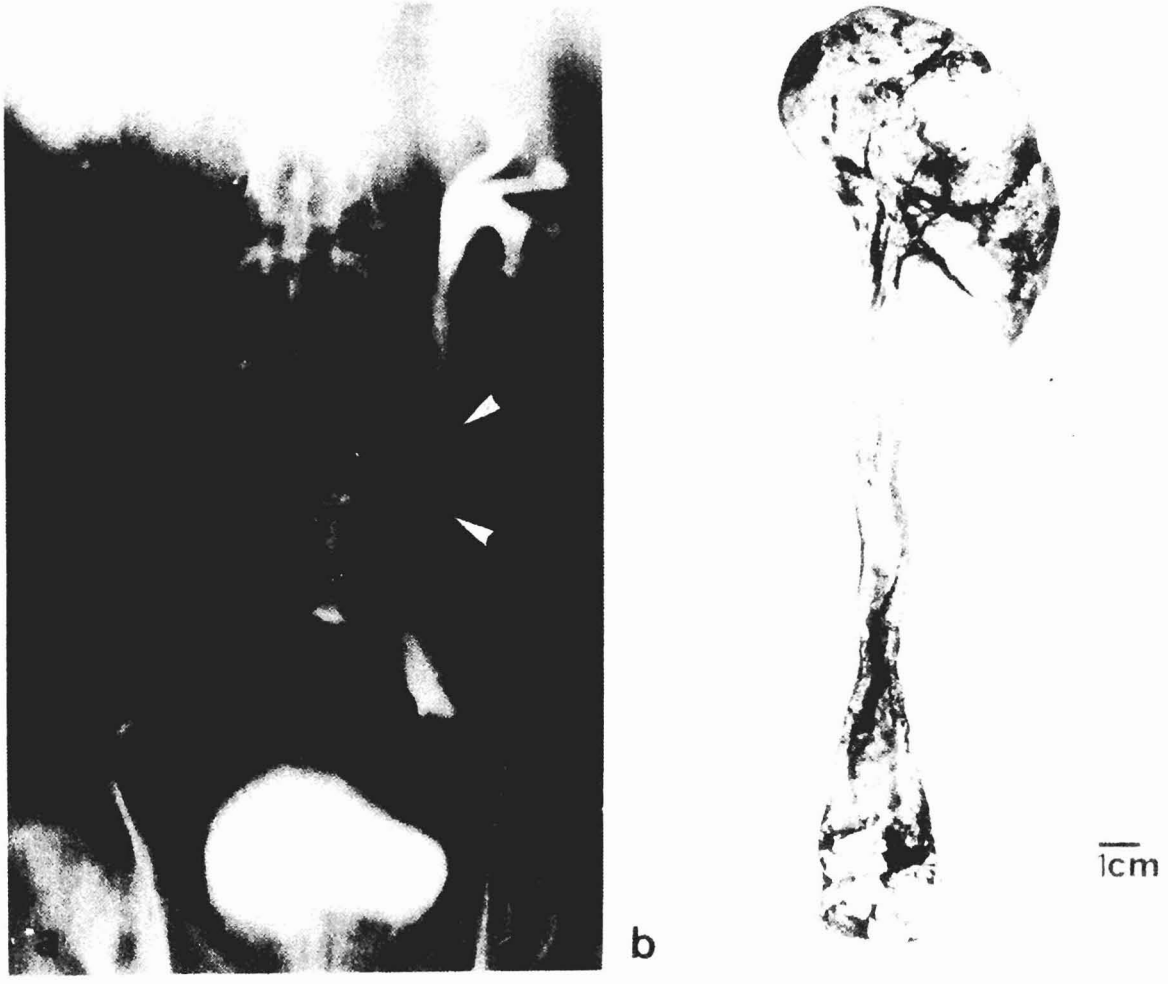

Fig. 1. a. Urography shows filling defect in the mid and distal portions of the left ureter. **b.** Gross pathological specimen.

endoplasmic organelles (Fig. 4b).

The superficial epithelial layers of papillary projections were arranged in a disorderly manner and the cells showed the following distinctive features: they had underdeveloped junctional complexes; the characteristic lateral cisternae were reduced in number; the nuclei were almost circular in cross section; the nuclei were not cleaved and there was no margination of the chromatin; and the endoplasmic organelles were scanty (Fig. 4a).

In the specimens examined by TEM, no nuclear pleomorphism nor any atypical mitotic figures were seen.

Observed by SEM at low power, the tumour presented a strking papillary pattern (Fig. 5a).

Tumour papillary branches had a superficial layer of cells which presented a cobblestone appearance and varied in size (Fig. 5b).

These superficial cells were generally covered by a surface membrane with variable numbers of bleb-like microvilli (Fig. 5c) and some of these cells possessed long pleomorphic microvilli (Fig. 5d). Normal superficial urothelial cells with an anastomosing system of microridges were not visible.

Discussion

In the observed case of papillary transitional cell carcinoma of the juxtavesical ureter, the most important

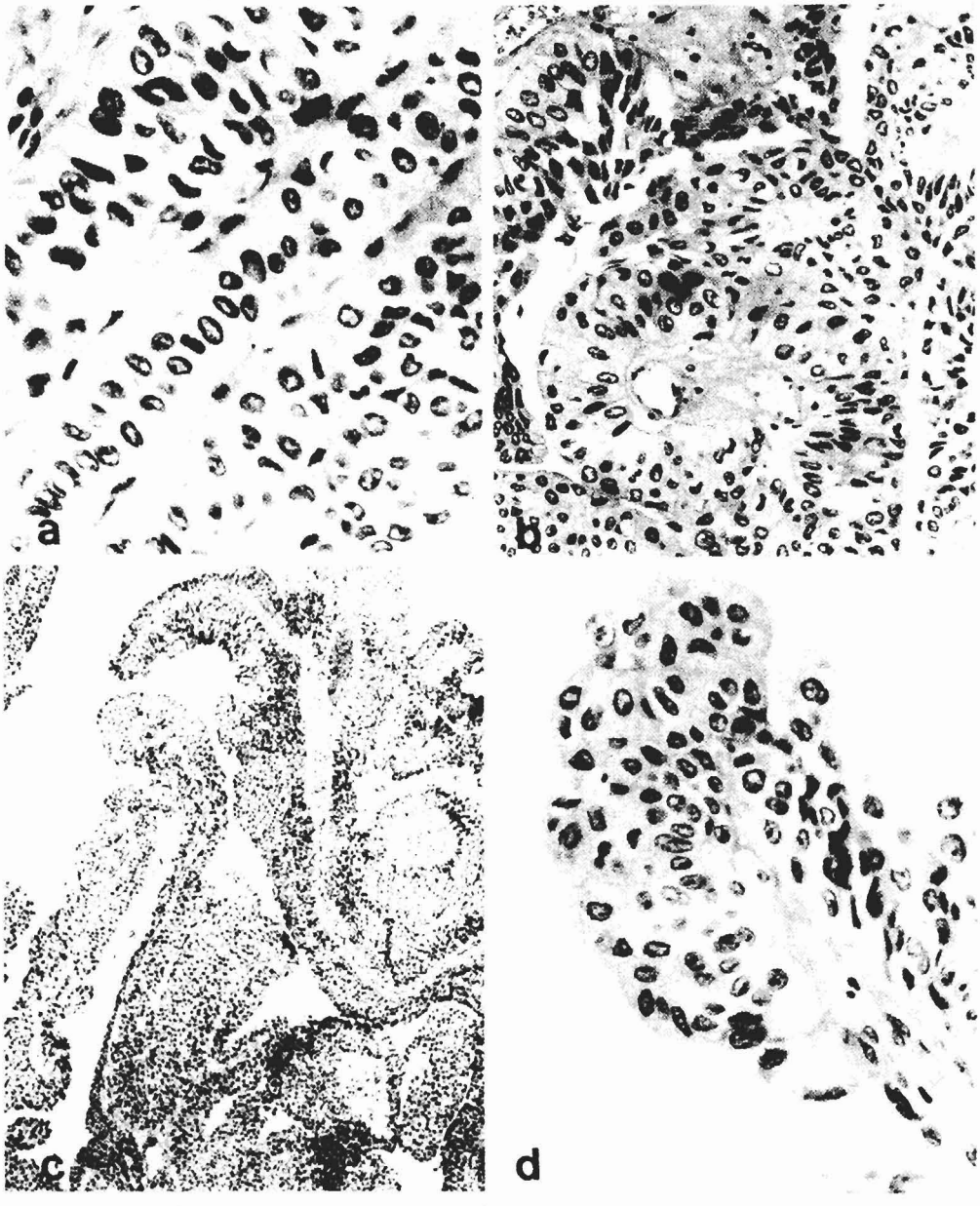

Fig. 2. a. Deep zone of neoplasm. Cellular cords with slender stroma. H-E, x 40. **b.** Neoplasm aspect in transitional zone towards papilliferous part. H-E, x 20. **c.** Superficial papilliferous zone. H-E, x 2,5. **d.** Detail of papilla apex. H-E, x 40

revelation was the high degree of differentiation of the tumour cells, not only in the various portions of the neoplasm but also in a solitary papilla itself.

Aspects of major cellular atypia were present in the most superficial layers and these became much more evident in the exfoliated cells collected in the intrapapillary recesses.

The dysplastic characteristics of the ureteral cells were present in the entire ureteral tract, below the neoplasm and for a certain distance above the neoformation.

In addition, ureteral dysmorphism and numerous Brunn nests were present. This could therefore be indicative of a relationship between these dysmorphic characteristics and the occurrence of the neoplasm.

This study, furthermore, confirms that the abnormal ultrastructural features of TCC of the ureter are comparable to those of TCC in the pelvis and the bladder. In fact, TEM observations confirmed the data of a majority of authors that the deep cells of the neoplasm are a normal morphological finding and that the superficial cells do not have the ultrastructural characteristics present in normal transitional cells, such as tripartite junctional complex, numerous cytoplasmic

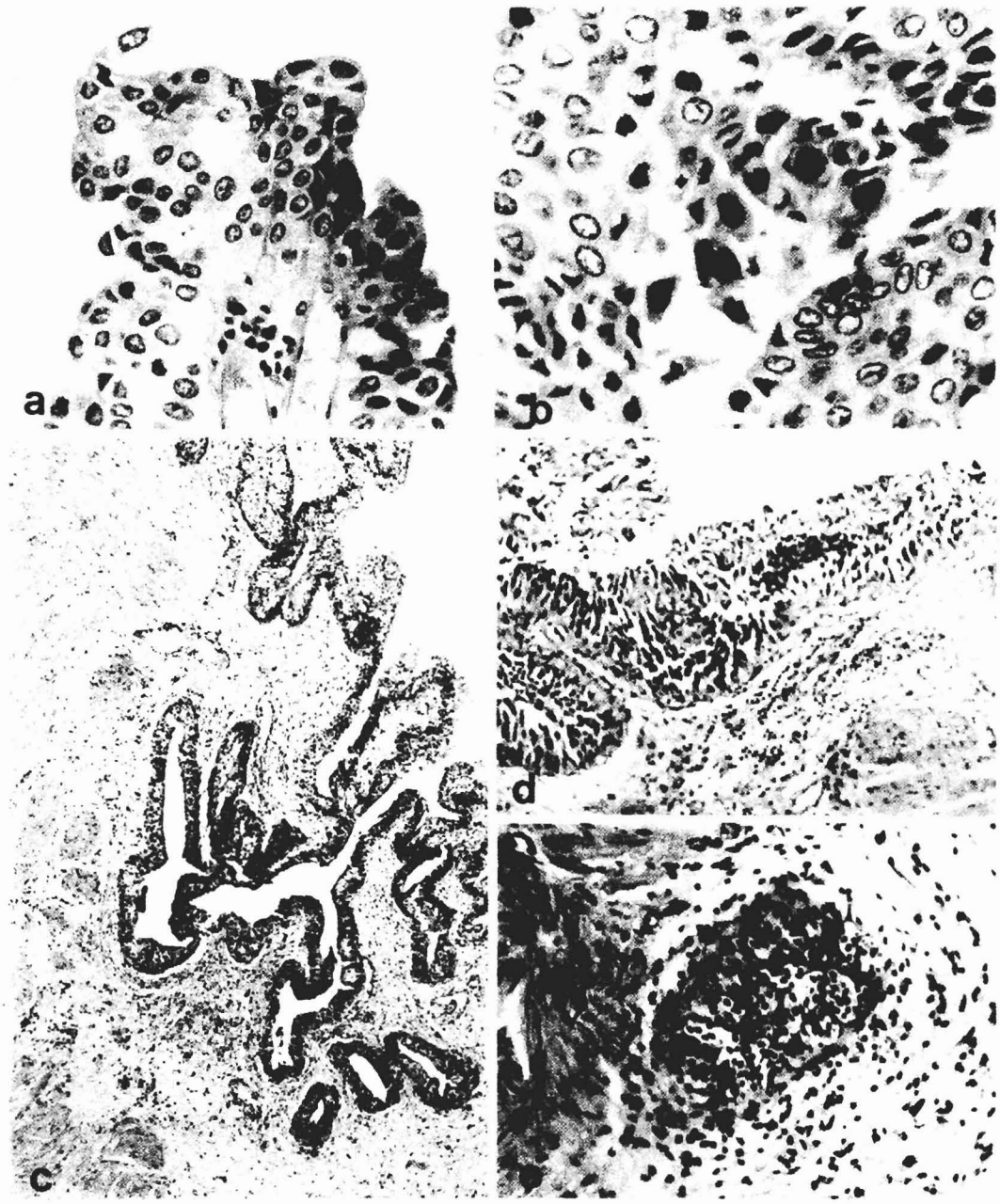

Fig. 3. a. Detail of papilla apex with atypical cells. H-E, x 40. **b.** Interpapillary zone of neoformation; note numerous desquamated atypical cells, variable in shape and size, with hyperchromic irregular nuclei and atypical mitoses. H-E, x 60. **c.** The most distal ureteral part from the neoplasm with severe dysplasia of epithelial cells. H-E, x 3.5. **d.** Ureteral mucosae adjacent to neoplasm. H-E, x 10). **e.** Presence of Brunn nests near neoplasm. H-E, x 10

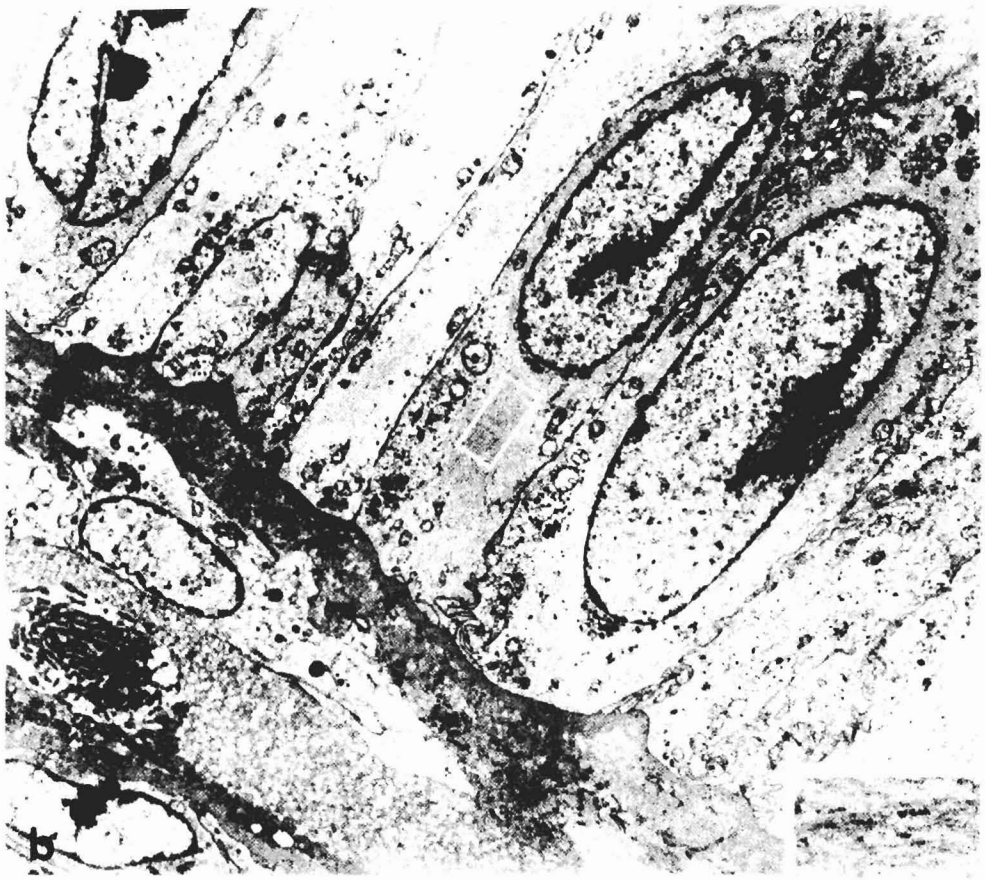

Fig. 4. a. Electron micrograph of apex papilla. Epithelial cells are disordely arranged. Their luminal surface contains microvilli (boxed area). The nuclei show a circular section without heterochromatin. TEM, x 4,800, boxed area TEM, x 10,000. The basal layer of papilla. The cells are oriented with their long axis parallel to the basement membrane. Note cleaved nuclei and numerous intercellular microfilaments (boxed area). TEM, x 5,000, (boxed area TEM, x 7,450

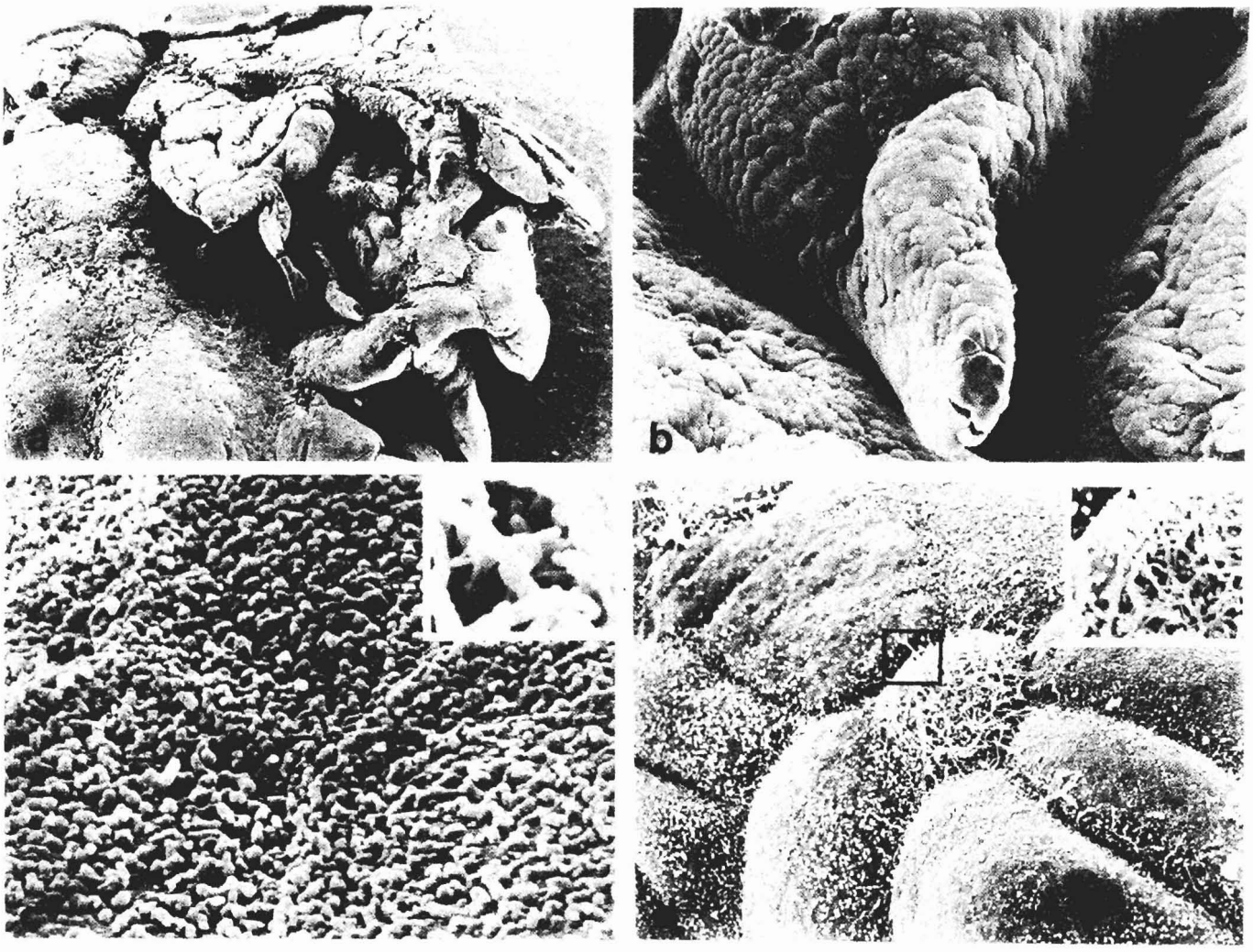

Fig. 5. a. Papillary pattern of the neoplasm. SEM, x 125. **b.** A papillary projection covered by cells with cobblestone appearance. SEM, x 180. **c.** Surface membrane with variable numbers of bleb-like microvilli. SEM, x 10,000, boxed area SEM, x 20,000. **d.** A cell with long pleomorphic microvilli (boxed area). SEM, x 1,800, boxed area SEM, x 5,000

organelles, oval nuclei with infoldings of nuclear membrane and margination of chromatin.

Moreover, the present morphological study suggests that the pathological changes in TCC follow an extremely complex topographical pattern, often involving small groups or even single urothelial cells.

SEM proved to be very useful for examining large areas of surface urothelium and the observations made were very helpful in formulating the above concept.

In fact, in our case, SEM showed the presence of small groups or single cells with «pleomorphic microvilli» on the cell surface. The presence of «pleomorphic microvilli» is regarded as a useful tumour marker by many authors who have observed these abnormalities in carcinomas of several organs, including mesothelial cells (Carpino et al., 1983), cervix (Jordan and Williams, 1971; Murphy et al., 1984), breast (Spring-Mills and Elias, 1975), colon (Kahan et al., 1976), liver (Ogawa et al., 1979), as well as transitional cell carcinomas of bladder and renal pelvis (Jacobs et al., 1981; Newman et al., 1988). In addition, the correlation between the pleomorphism of microvilli and the grade of the tumour (Tannenbaum et al., 1978; Jacobs et al., 1981; Newman et al., 1988) concurred with light microscopy observations to confirm a grade 2 to our tumour.

References

Abeshouse B.S. (1956). Primary benign and malignant tumors of the ureter. A review of the literature and report of 1 benign and 12 malignant tumors. Am. J. Surg. 91, 237-240.

Batata M.A. and Grabstald H. (1976). Upper urinary tract urothelium tumors. Urol. Clin. North. Ann. 3, 79.

Batata M.A., Whitmore W.F. jr, Hllarir B.S., Tokita N. and Grabstald H. (1975). Primary carcinoma of the ureter: a prognostic study. Cancer

35, 1625-1628.

Bengtsson U. (1962). Comparative study of chronic non obstructive pyelonephritis and renal papillary necrosis. Acta Med. Scand. (suppl.), 388-390.

Bengtsson U., Angervall L. and Johansson S. (1973). Phenacetin abuse and renal pelvic carcinoma. In: Problem des phenacetinabusus. Haschek H. (ed). Facta publication Vienna. Vienna. pp 221-226.

Bennington J.L. and Beckwith J.B. (1975). Tumours of the kidney, renal pelvis and ureter. In: Atlas of tumour pathology. Series 2, Fascicle 12. Armed Forces Institute of Pathology. Washington DC. pp 255-263 and pp 304-310.

Buch H., Hauser H., Pfleger K. and Rudger W. (1966). Bestimmung von phenacetin und N-acetyl-p-aminophenol uber stoff wech-sel produkte im harn. Z. Klin. Chem. Klin. Biochem. 4, 288-290.

Cameron K.M. (1969). Editoriale. Proc. R. Soc. Med. 62, 96-100.

Carpino F., Paggiarino D., Gaudio E., Pericoli M.N., De Santis M., Migliore G. and Melis M. (1983). A comparative light and scanning electron microscopic study for identification of atypical human cells. Giornale Italiano Oncologia 1, 9-13.

Das A.K., Carson C.C., Bolick D. and Paulson D.F. (1990). Primary carcinoma of the upper urinary tract. Cancer 66, 1919-1923.

Eagan J.W. jr (1989). Urothelial neoplasms: urinary bladder. In: Hillgs uropathology. Churchill Livingstone. New York. pp 793-841.

Grabstald H., Whitmore W.F. and Melaned M.R. (1971). Renal pelvic tumors. JAMA 218, 845-854.

Highman W. (1986). Transitional carcinoma of the upper urinary tract: a histological and cytopathological study. J. Clin. Pathol. 39, 297-305.

Hvidt V. and Feld-Rasmussen K. (1973). Primary tumors in the renal pelvis and ureter with particular attention to the diagnostic problems. Acta Chirs. Scand. (suppl) 433, 91-101.

Jacobs B.J., Chen S.M., Farrow G.M. and Friedell G. (1981). Scanning electron microscopic features of human urinary bladder cancer. Cancer 48, 1399-1409.

Jensen O.M., Knudsen J.B., Tomasson H. and Sorensen B.L. (1989). The Copenhagen case-control study of renal pelvis and ureter cancer. Role of analgesics. Int. J. Cancer 44, 965-968.

Jordan J.A. and Williams A.E. (1971). Scanning electron microscopy in the study of cervical neoplasia. J. Obstet. Gynaecol. Commonwealth 78, 940-946.

Kahan B.D., Rutsky L. and Berlin B. (1976). Cell surface alterations on colon adenocarcinoma cells. Cancer Res. 36, 3526-3534.

Luft J.M. (1961). Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9, 409-410.

Mahadevia P.S., Karva G.L. and Koss L.G. (1983). Mapping of urothelium in carcinomas of the renal pelvis and ureter. Cancer 51, 890-895.

Murphy J.F., Jordan J.A., Allen J.H. and Williams A.E. (1974). Correlation of scanning electron microscopy, colposcopy and histology in 50 patients presenting with abnormal cervical cytology. J. Obstet. Gynaecol. Br. Commonwealth 81, 236-241.

Newman J., Antonakopoulos G.N. and Hicks R.M. (1988). Scanning electron microscopy of the upper urinary tract in transitional cell carcinoma of the renal pelvis. Br. J. Exp. Pathol. 69, 525-536.

Ogawa K., Medline A. and Farber E. (1979). Sequential analysis of hepatic carcinogenesis. A comparative study of the ultrastructure of preneoplastic, malignant, prenatal and regenerating liver. Lab. Invest. 41, 22-35.

Petkovic S.D. (1975). Epidemiology and treatment of renal pelvic and ureteral tumors. J. Urol. 114, 858-860.

Petkovic S.D. (1981). Epidemiology and treatment of high grade transitional cell cancer of the upper urinary tract. J. Urol. 125, 25-27.

Poole-Wilson D.S. (1960). Occupational tumors of the bladder. Proc. R. Soc. Med. 53, 801-805.

Reynolds E.S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17, 208-210.

Schade R.O.K. and Swinney S. (1968). Precancerous changes in bladder epithelium. Lancet 2, 943-945.

Sharma T.C., Melamed M.R. and Whitmore W.F. jr (1970). Carcinoma in situ of the ureter in patients with bladder carcinoma treated by cystectomy. Cancer 26, 583-587.

Spring-Mills E. and Elias J.J. (1975). Cell surface differences in ducts from cancerous and non cancerous human breasts. Science 188, 947-949.

Steffens J. and Nagel R. (1988). Tumours of the renal pelvis and ureter observations in 170 patients. Br. J. Urol. 61, 277-283.

Tannenbaum M., Tannenbaum S. and Carter H.W. (1978). SEM, BEI and TEM ultrastructral characteristic of normal, preneoplastic and neoplastic human transitional epithelia. Scan. Electron Microsc. II, 949-956.

Watson M.L. (1958). Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. 4, 475-477.

Whitmore W.F. jr (1978). Bladder Cancer. Cancer 28, 170-175.

Accepted January 10,1994