# Ultrastructure of Renal-Cell Tumors Induced in Mice by 1,2-Dimethylhydrazine

V. F. Kondalenko, G. Yu. Chemeris, and V. S. Turusov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 9, pp. 286-290, September, 1995
Original article submitted October 19, 1994

Fifteen primary renal adenomas induced with 1,2-dimethylhydrazine in CBA mice and 3 passages of adenomas transplanted subcutaneously were examined by electron microscopy. A well-developed brush border was found in one primary adenoma, this being in line with histochemical data on the presence of markers of the proximal canaliculi epithelium in this tumor. In other tumors a reduced brush border (5 cases) and apical canaliculi (14 cases) were revealed, which are typical of the proximal canaliculi epithelium. The ultrastructure of the types of adenoma cells is described. Atypical tumor cells with intracellular lumina were found in transplanted adenomas

**Key Words:** *renal-cell tumors of mice; ultrastructure; cell types*

The possibility of renal-cell tumor (RCT) histogenesis from stem cells has been discussed more than once [1,5]. The capacity of stem cells and of renal canaliculi epithelium precursor cells for differentiation in highly specialized elements of the proximal, fine, and distal segments of the nephron allows us to assume that RCT could develop according to the histological characteristics of each canaliculus. Characteristic features of the epithelium of the proximal or distal canaliculi [2,7-9,13] and collecting tubules [12, 13] can be detected in human and animal RCT.

Previously we presented histochemical and immunohistochemical data on the histogenesis of 1,2-dimethylhydrazine (DMH)-induced renal adenomas in CBA mice [2,13]. Only one out of 48 tumors examined possessed features of the proximal canaliculi epithelium. All the others showed homology of tumor cells to the distal canaliculi or collecting tubule epithelium [2,13]. This prompted us to investigate the ultrastructure of such tumors. At the submicroscopic level, there are clear-cut criteria for identification of the epithelium of the proximal canaliculi: brush border, numerous tubular invaginations (apical canaliculi) in the apical regions of the cytoplasm, and associated vacuoles of varying size and electron density [4].

Department of Carcinogens, Research Institute of Carcinogenesis, Cancer Research Center, Russian Academy of Medical Sciences, Moscow (address for correspondence: 24 Kashirskoe shosse, 115478, Moscow) (Presented by Yu. N. Solov'ev, Member of the Russian Academy of Medical Sciences)

## MATERIALS AND METHODS

Fifteen RCT of CBA mice used in previous studies [2,13] were examined under the electron microscope. In addition, 3 passages of a primary tumor (illustrated in our earlier papers [2,13]) subcutaneously transplanted to syngeneic mice were examined. Primary RCT were induced by repeated subcutaneous injection of DMH in a dose of 8 mg/kg [2,13]. The material was fixed in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide in the same buffer, and embedded in an epon-araldite mixture. Three tumors were obtained from histopathological archives; two of them were stored in neutral formalin and one in castor oil.

## RESULTS

Our previous histochemical and immunohistochemical studies had demonstrated markers of the dis-

0007-4888/95/0009-0925$12.50 

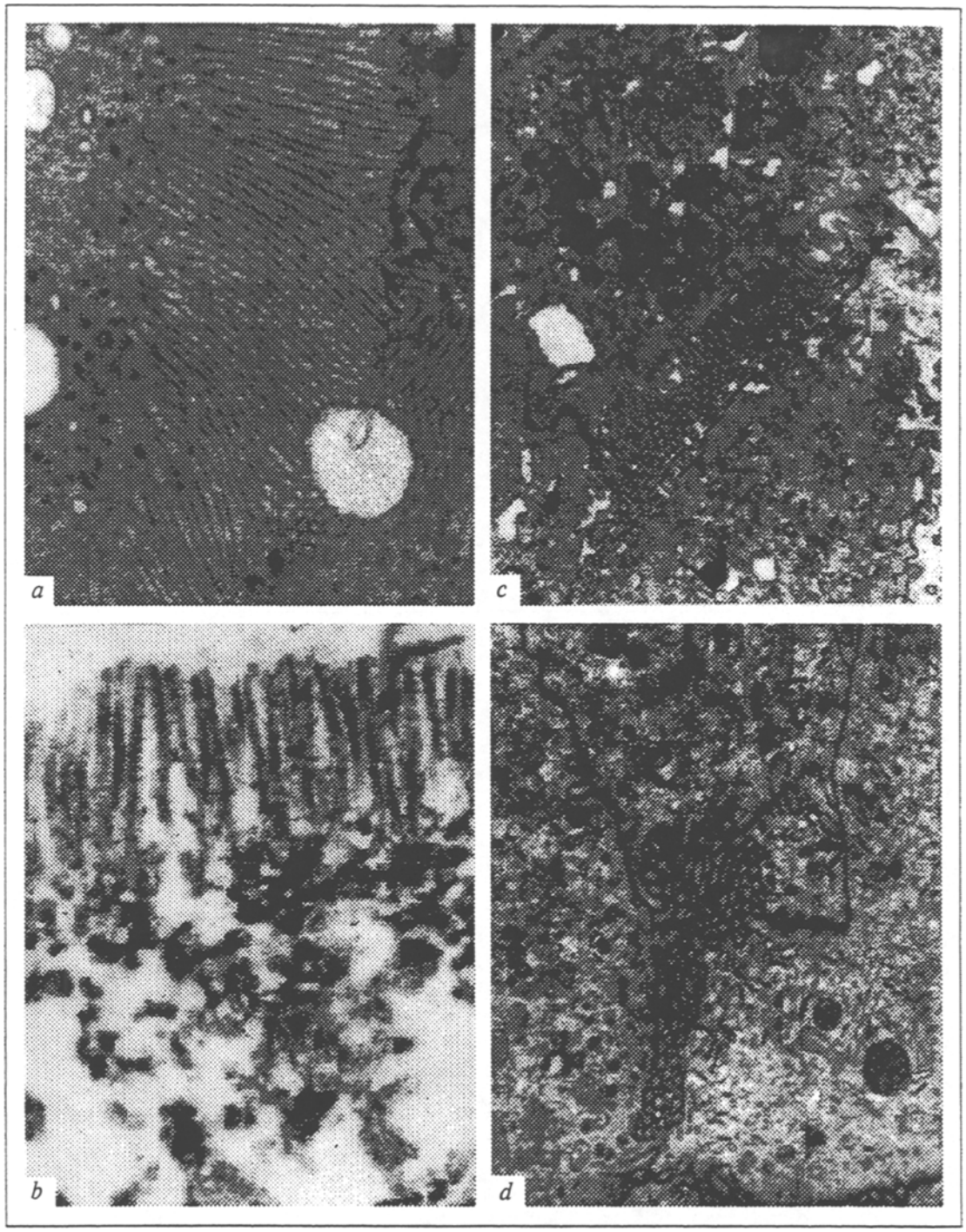


**Fig. 1.** Ultrastructure of tumor cells of experimental renal adenomas in mice. *a*) brush border of the proximal canalicular epithelium in intact murine kidney; long microvilli tightly adhere to one other; the apical part of the cytoplasm contains apical canaliculi and numerous small vacuoles. ×15,000; *b*) brush border in adenoma with histochemically detected markers of the proximal canalicular epithelium; microvilli are seen on the entire apical surface of the epithelium despite the poor condition of the material obtained from archives. ×30,000; *c*) reduced brush border in adenoma, microvilli cut obliquely and crosswise. ×15,000; *d*) reduced brush border in subcutaneously transplanted renal adenoma, second passage. ×20,000; *c*, *d*) adenomas with histochemical markers of the distal canaliculi.

tal canaliculi or collecting tubule epithelium in all studied tumors but one, whereas markers of the proximal canaliculi were absent [2,13]. Despite the fact that the ultrastructure of this one archival tumor with markers of the proximal canaliculi epithelium was not well preserved, electron microscopy showed a distinct brush border. Normally the brush border of the proximal canaliculi is rather

high (Fig. 1, *a*). In the studied tumor it was somewhat lower but, as in health, microvilli covered the entire apical surface of cells, adhering quite closely to each other (Fig. 1, *b*). In five other tumors with immunohistochemical markers of the distal canaliculi microvilli covered just some areas of the apical surface of tumor cells, being absent or sparse in other places. Slitlike lumina into which the reduced brush border was directed were seen in the same tumors (Fig. 1, *c*). This border consisted of a few closely adhering microvilli (Fig. 1, *c*). A reduced brush border was found in the second passage of the transplanted tumor, which corresponded to the distal canaliculi in immunohistochemical parameters (Fig. 1, *d*).

In the examined tumors we found tubular invaginations and small vacuoles, which are typical of the apical regions of the proximal canaliculi epithelium in health; these structures were seen in cells both with and without reduced brush border. In some cases they were numerous, filling an appreciable part of the cytoplasm (Fig. 2, *a*). The contents of tubular invaginations are, as a rule, electron-transparent. The lumen of invaginations was frequently narrowed in the upper part, so that they acquired a dumbbell-like or club shape. Such formations may also be encountered in the normal epithelium of the proximal canaliculi. The causes of the marked increase of their number in the cytoplasm of renal adenomas merit special study.

The histogenesis of renal adenomas and cancer is still unknown. The hypothesis that RCT originate mainly from the proximal canalicular epithelium [7,11] has had to be rejected. It has been demonstrated in many studies that, according to their characteristics, tumor cells may correspond to the epithelium of both the proximal and distal canaliculi and collecting tubules [2,8,12,13]. This conclusion is based on studies of tumor ultrastructure and histochemical and immunohistochemical features. For example, nitrosomorpholine injected to rats was found to induce renal tumors possessing the histological characteristics of the epithelium of not only the proximal canaliculi, but also the distal canaliculi and collecting tubules [3].

The presence of tumor cells of differently directed differentiation in the tumors we studied is not easy to explain. According to the concept of RCT origination from precursor cells, their daughter cells should to acquire the antigenic and morphological characteristics of different portions of the nephron in the same tumor. Another variant is possible: due to tumor transformation, abnormal gene expression may be observed in tumor cells, and in such a case cells with signs of epithelium of the proximal and distal canaliculi and the collecting tubules may occur in one and the same tubular structure of the tumor [8].

Chromophobic, basophilic, acidophilic, and clear cells are distinguished in renal adenomas of rats [3,6]. Renal tumors consisting of oncocytes have also been observed in rats [3]. A specific tumor cell type is shown to correspond to each nephron compartment [3].The proximal canaliculi give rise mainly to basophilic-cell tumors in rats, whereas the clear-cell variant of tumors and oncocytomas probably originates from the distal canaliculi and collecting tubules [3]. All cell variants of tumors observed experimentally have been found in humans as well [3].

All the cell types mentioned, except oncocytes, were detected at the ultrastructural level in renal adenomas of mice. Numerous vacuoles were seen in the cytoplasm of chromophobic cells. The cytoplasm of basophilic cells was filled mainly with free polysomes and canaliculi of granular cytoplasmic reticulum, whereas the cytoplasm of acidophilic cells contained numerous mitochondria. One type of clear cells was frequently observed in the tumors we examined. Their cytoplasm contained a large quantity of glycogen granules (Fig. 2, *b*) or accumulations of lipid corpuscles (Fig. 2, *c*), or both. Such cells were described in detail for rat RCT [3]. The appearance of this type of clear cells seems to be caused by disturbed energy metabolism in the tumors [3].

The transplanted murine RCT which we examined frequently contained well-differentiated cells with rather large numbers of mitochondria in the cytoplasm and canaliculi of the granular cytoplasmic reticulum. Atypical tumor cells were found in the same tumors (Fig. 2, *d*). Homogeneously distributed uncondensed chromatin was seen in the nucleus and very few organelles in the cytoplasm. In addition, there were cells with intracellular lumina in the cytoplasm. A few microvilli could be discerned on the surface of these lumina. Such intracellular gaps may occur in tumors of different origin and may be indicative of increasing malignancy [10]. In our experiments transplantation of the renal adenomas probably led to the increase of tumor malignancy, as was confirmed by the appearance of atypical tumor cells and intracellular lumina in the transplants.

The immunohistochemical and electron-microscopic data were in complete agreement in only one of the tumors we observed; this tumor yielded a positive response to γ-glutamyltranspeptidase, a marker of the proximal canaliculi, and a negative reaction to A6 antigen, a marker of the distal

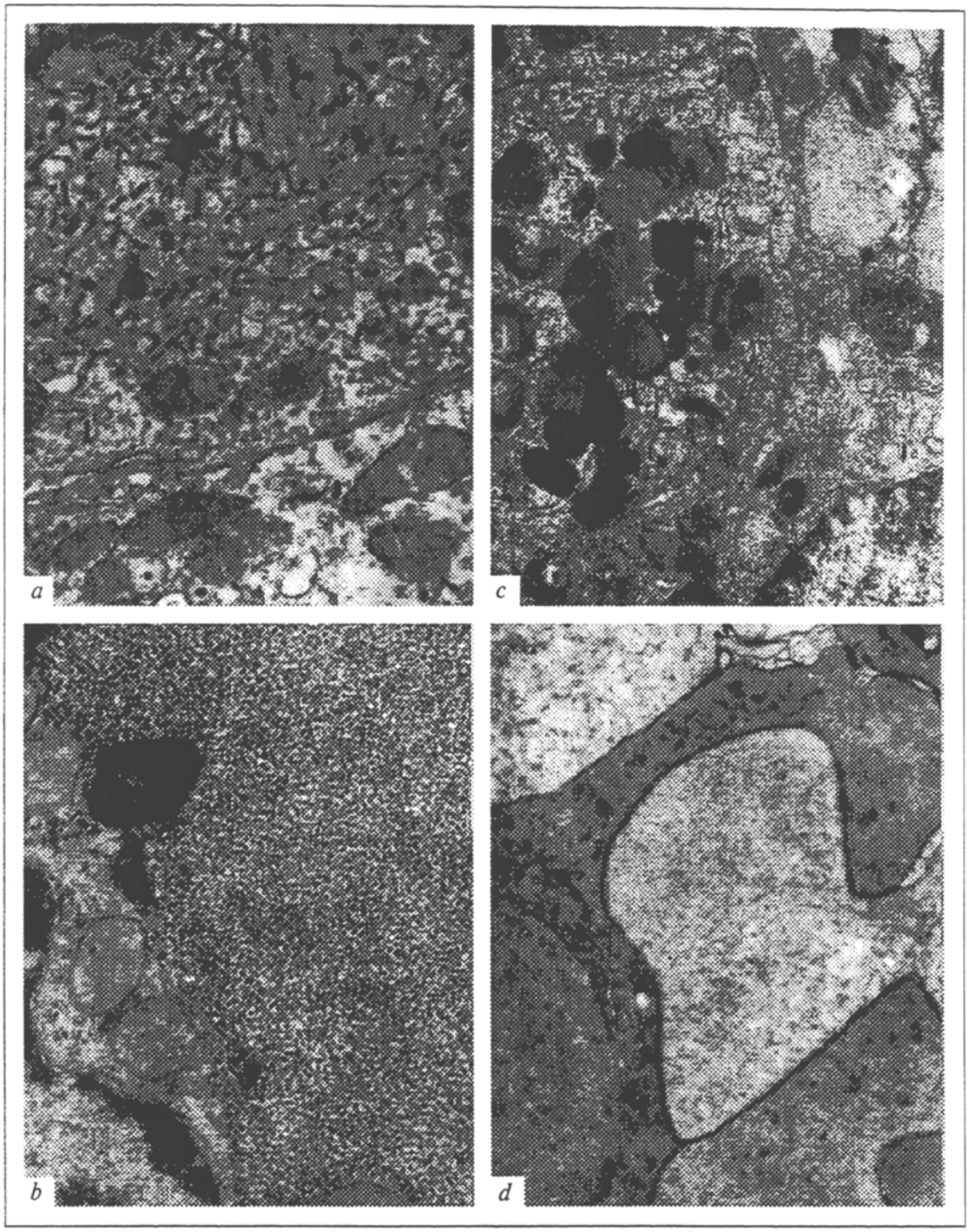


**Fig. 2.** Ultrastructure of tumor cells of experimental renal adenomas with histochemical markers of the distal canaliculi. *a*) numerous structures similar to apical canaliculi in the cytoplasm. ×20,000; *b*) accumulation of glycogen particles in the cytoplasm of a clear tumor cell. ×40,000; *c*) lipid corpuscles in the cytoplasm of a clear tumor cell. ×30,000; *d*) atypical tumor cells with atypically shaped processes in transplanted renal adenoma, second passage. ×20,000.

canaliculi and collecting tubules. A well-developed brush border was detected at the ultrastructural level (Fig. 1, *b*). No agreement of this kind was seen in the rest of the tumors examined: all the tumors were A6-positive and responded negatively to γ-glutamyltranspeptidase, but electron-microscopic study revealed a brush border, albeit a reduced one, in many of them.

## REFERENCES

1. N. T. Raikhlin and Yu. L. Perov, *Arkh. Pat.*, № 11, 41-49 (1980).

2. G. Yu. Chemeris, V. S. Poltoranina, and V. S. Turusov, *Ibid.*, № 2, 48-52 (1992).
3. P. Bannasch and H. Zerban, in: *Tumors and Tumor-Like Conditions of the Kidneys and Ureters,* Ed. J. N. Eble, New York - Edinburgh - Melbourne (1990), pp. 1-34.
4. W. Bloom and D. W. A. Fawcett, *Textbook of Histology,* 9th ed., Philadelphia - London - Toronto (1969).
5. C. Cohen, P. A. McCue, and P. B. Derose, *Cancer,* **62**, 1946-1961 (1988).
6. D. R. Dietrich and J. A. Swenberg, *Mutat. Res.,* **248**, 239-260 (1991).
7. E. R. Fisher and E. Howat, *Urology,* **108**, 384-386 (1972).
8. P. Holm-Nielsen and T. S. Olsen, *Ultrastruct. Pathol.,* **12**, 27-39 (1988).
9. L. Radovic, D. Fergula, A. Masera, and Z. Oveak Kregar, *J. Histochem. Cytochem.,* **10**, 200-201 (1988).
10. L. Remy, *Biol. Cell,* **56**, 97-100 (1986).
11. R. Seljelid and J. Ericsson, *Lab. Invest.,* **14**, 435-447 (1985).
12. S. Storkel, P. V. Steart, D. Dreneckhahn, and W. Hoenes, *Virchows Arch. [B],* **56**, 237-245 (1989).
13. V. S. Turusov and G. Yu. Chemeris, *Toxicol. Pathol.,* **2**, 570-575 (1992).