

Ultrastructure and Nanomorphology of the American Mink (*Mustela vison*) Kidney

V. O. Ezhkov^a, M. S. Ezhkova^b, I. A. Yapparov^a, A. Kh. Yapparov^a, I. R. Nizameev^b,
E. S. Nefed'ev^b, A. M. Ezhkova^{a,*}, and Yu. V. Larina^a

Presented by Academician A.Ya. Samuilenko August 1, 2018

Received December 24, 2018; revised December 24, 2018; accepted December 24, 2018

Abstract—The ultrastructure of the nephron subcellular organelles was studied in healthy mink kidneys. The data obtained were compared with the results of transmission electron microscopy. The renal cell nanomorphology proved to be similar when electronograms and the atomic force microscopy images were analyzed. The methods used enabled us to visualize the glomerular capillary endotheliocytes with cytolemma pits in the area of fenestrae that provide blood filtration; in the proximal nephron part, on the apical pole of the epithelial cells, brush-border soft microvilli were observed. The microvilli were characterized by a well-organized structure along their entire length and the membrane integrity. The data obtained show morphological parameters of the healthy mink organ and can be helpful in diagnosing of nephropathology.

DOI: 10.1134/S0012496619020091

The kidney is a paired organ that regulates the body chemical homeostasis through the function of urine formation. The kidney plays the leading role in the urinary excretion of vertebrates, including humans. The following kidney functions are known: excretory, homeostatic, metabolic, incretory and protective. Urine formation and excretion is the main kidney function of the body excretory system. The nephron is a structural and functional unit of the kidney; it consists of a glomerulus of capillaries, glomerular capsule (renal corpuscle), and a system of tubules [1].

To obtain unbiased morphological evidence when normal ontogeny is studied or clinical diagnosis is verified, the qualitative and quantitative analyses of the biopsy and autopsy materials are required, such as the light optical, electron microscopic and nanomorphological analyses. Because of active nanotechnology introduction into different fields of biology and veterinary medicine, the fundamental structure of animal organs and tissues can be studied at the nanoscale level. The main instrument of this kind of studies is the scanning probe microscope [2].

Data on the organ ultrastructure and nanomorphology are scanty and the contradictory interpretations of them are given by different authors, though these studies are important for evaluation of the structural and functional kidney state. The use of modern methods and devices makes it possible to clarify a series of issues of the fundamental morphology and to obtain evidences at the different levels of the living material organization.

The objective of this study was to investigate the ultrastructure and nanomorphology of kidney cells from the organs of clinically healthy minks with the use of transmissive electron microscopy and atomic force microscopy. In this way, we have studied the structural and functional state of a glomerular filter and the epithelial cells of the nephron proximal part.

The material studied were kidneys of the seven-month-old clinically healthy young minks grown at the Bersutsky Agrofirma (Russia). The organs of 36 minks were withdrawn during the period of technological slaughter of the fur animals [3].

When studying the organ structure by the method of transmission electron microscopy, kidney plates $0.1 \times 0.1 \times 0.1$ cm in size were placed into purified 2.5% glutaraldehyd solution and cut into 0.1-mm^3 pieces before fixation for 4–6 h. After the next fixation for 2 h in the osmium solution at 4°C , the kidney pieces were embedded in araldite–epon. Kidney sections obtained on a LKB-III ultratome (LKB, Sweden) were contrasted for 15 min using 2% ethanol solution of uranyl acetate and Reynold's lead citrate.

^a Tatar Research Institute for Agricultural Chemistry and Soil Science, Kazan Scientific Center, Russian Academy of Science, Kazan, 420059 Tatarstan, Russia

^b Kazan National Research Technological University, Kazan, 420015 Tatarstan, Russia

* e-mail: egkova-am@mail.ru; niixp2@mail.ru

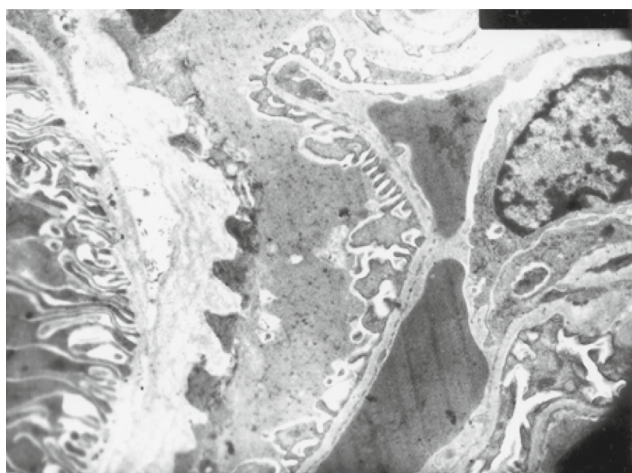


Fig. 1. Ultrastructure of the smooth-muscle type mesangial cells in a glomerule of the inner cortical renal corpuscle that interacts with the capillary basal membrane; $\times 8000$.

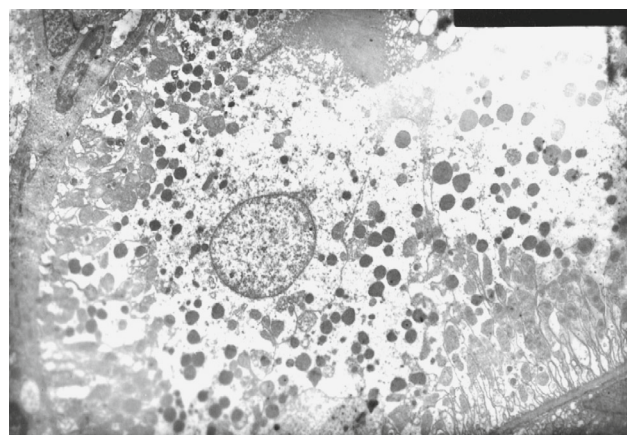


Fig. 2. Ultrastructure of the convoluted tubule epitheliocytes of the nephron proximal part with the pronounced basal labyrinth and numerous cigar-like mitochondria between its membranes; $\times 5000$.

The preparations were examined under a JEM-100CX electron microscope (JEOL, Japan) at an accelerating voltage of 75 kV. The search and identification of the required parts of the organ were performed using an array of ultrathin sections.

To study the kidney nanomorphology by the method of atomic force microscopy (AFM), organ pieces $0.1 \times 0.1 \times 0.5$ cm in size were fixed in 10% aqueous solution of neutral formalin (9 : 1) at a temperature of 4°C for a week. Afterwards, the material was washed with running water for 30 min. In the preparations fixed on the metal substrate, measurements were performed using a scanning MultiMode V probe microscope (VEECO, United States) in the mode of intermittent contact atomic force microscopy. RTESP rectangular cantilevers (VEECO) with silicone probes were used for scanning. The resonant frequency of the cantilever was 250–350 kHz, the probe curvature radius was 10–13 nm. The preparations were analyzed at a scanning speed of 1 Hz and image resolution of 512×512 pixels.

The most typical areas of kidney with repetitive relief were selected to examine the renal microstructure. In a glomerular filter of the renal corpuscles, contacts were observed between mesangial cells of the smooth-muscle and mononuclear types and the basal membrane of the glomerular capillaries (Fig. 1). At the same time, blood filtration occurred, as well as the primary urine formation, which was followed by its excretion into the nephron capsule. The electron-microscopic examination showed a well-formed basal labyrinth and numerous cigar-like mitochondria between its membranes in epitheliocytes of the convoluted tubules in the proximal part of the nephron (Fig. 2). The epithelial cells of the nephron proximal tubules have distinct microvilli, which form the brush border (BB) at the apical pole. The basal poles of the epithe-

lial cells of the renal proximal tubules were in contact with full-blooded vessels.

Atomic force microscopy provides the opportunity to study the topography and morphology of the living cells, interactions between cells, and subcellular structures at the atomic level [4–7].

In the AFM images, the glomerular capillary endotheliocytes of the renal corpuscles have the cytolemma pits characteristic of fenestrae of these cells to provide blood filtration (Fig. 3). We have imaged the soft structures of the BB microvilli on the apical pole of the epithelial cell of the proximal nephron part. Well-structured BB without any damages of the microvilli

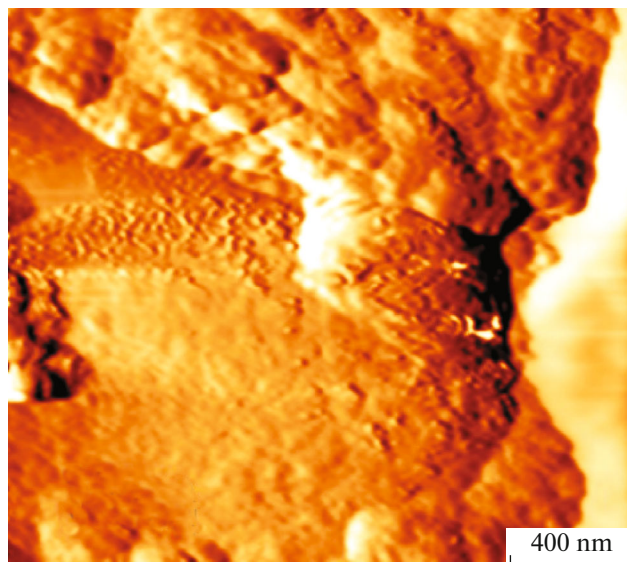


Fig. 3. AFM image of the glomerular capillary endotheliocyte with the cytolemma pits in the fenestra region.

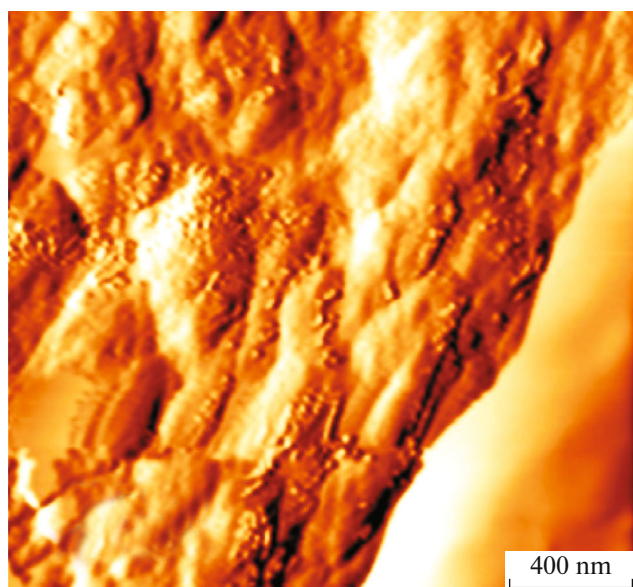


Fig. 4. AFM image of the brush border microvilli of the endotheliocyte of the renal nephron proximal tubule.

membrane surface along the entire length up to the end can be well seen in the AFM images (Fig. 4).

Thus, we have used electron microscopy to study the ultrastructure and obtained the nanomorphological AFM images of some subcellular components of the renal nephrons of clinically healthy minks. Comparative analysis of the data obtained by these two methods showed correspondence between AFM images and electronograms. The AFM method used for imaging of the renal structures proved to be less laborious and rather informative. Our data on the

mink kidney nanomorphology make it possible to determine the morphological parameters of healthy organ. On the basis of these parameters, AFM imaging of the kidney nanomorphology can be helpful in early diagnosis of nephropathy.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. We adhered to the international recommendations (Code of Ethics) for handling animals used in medical and biological research [3].

REFERENCES

1. *Fiziologiya cheloveka* (Human Physiology), Pokrovskii, V.M. and Korot'ko, G.F., Eds., Moscow: Meditsina, 2003.
2. Pavlovich, E.R., *Fundam. Issled.*, 2008, no. 6, pp. 107–108.
3. www.msu.ru/bioetika/doc/recom.doc
4. Ezhkov, V.O., Ezhkova, A.M., Yapparov, A.Kh., Yapparov, I.A., Nizameev, I.R., and Nefed'ev, E.S. *Ross. Nanotekhnol.*, 2017, vol. 12, nos. 7–8, pp. 107–113.
5. Schillers, H., Medalsy, I., Hu, S., et al., *J. Mol. Recogn.*, 2016, vol. 29, no. 2, pp. 95–101.
6. Chiou, Y.W., Lin, H.K., Tang, M.J., et al., *PLoS One*, 2013, vol. 8, no. 10, e77384.
7. Wyss, H.M., Henderson, J.M., Byfield, F.J., et al., *Am. J. Physiol. Cell Physiol.*, 2011, vol. 300, no. 3, pp. 397–405.

Translated by A. Nikolaeva