ELECTRON-MICROSCOPIC STUDY OF THE CYTOSKELETON OF HUMAN PODOCYTES

V. B. Zaitsev, N. T. Raikhlin, and I. N. Sokolova

UDC 591.461.3:597.562+611.018.1.086.3

KEY WORDS: podocyte; cytoskeleton; microtubules; microfilaments; intermediate filaments

In the modern view, renal ultrafiltration in vertebrates is an active process in which the cytoskeleton of the podocytes plays an important regulatory role [5, 6]. An electron-microscopic investigation of the structure of the podocyte cytoskeleton of representatives of the principal classes of vertebrates, including more than 30 species, was undertaken previously [1-3]. It was found that the podocyte cytoskeleton in the mammalian kidney consists of microtubules and networks of microfilaments, whereas in lower vertebrates it consists mainly of bundles of intermediate filaments. The ultrastructural components of cells of different organs and tissues, located far from regions affected by a tumor, are known not to differ from those in normal organs and tissues either quantitatively or qualitatively. Some workers [9] accordingly consider that intact regions of kidney tissue can be used as an object for morphological study of the cytoskeleton of normal epithelial cells of the human kidneys (for example, during surgical removal of a hypernephroma).

The aim of this investigation was a comparative ultrastructural study of the matrix of the podocytes in human renal tubules from the age aspect.

EXPERIMENTAL METHOD

Pieces of tissue from the intact zone of the kidney of patients with a Wilms' tumor, aged, 2, 4, and 6 years, and with carcinoma of the kidney, aged 37 and 65 years (six cases altogether) were investigated. Pieces of tissue were taken to allow for subsequent differential study of superficial, intercortical, and justaglomerular tubules. After fixation with glutaraldehyde and dehydration in alcohols the material was embedded in Epon-812. Semithin and ultrathin sections were stained with toluidine blue and lead citrate respectively. The sections were studied in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The podocyte cytoskeleton in the kidneys of children aged 2, 4, and 6 years was identical in structure. One of its main components is a system of microfilaments (Fig. 1a). The latter, 6-7 nm thick, joined and intersect each other to form a dense, widely branched network, penetrating throughout the cytoplasm of the podocytes and their processes. Ribosomes are usually found in the zones of intersection of individual microfilaments. It is suggested that these ribosomes consist of messenger RNA which, by establishing communication between nucleus and cytoskeleton, monitor the motor responses of the cells, which are effected through a locomotor apparatus [4]. Stratified bundles of polysomes, from which emerge large numbers of microfilaments, evidently synthesized there (Fig. 1b, c), are often found in the cytoplasm of the podocytes (Fig. 1b, c). The latter are formed in the cytoplasm by polymerization from G-actin. The network of microfilaments has intimate topographic relations also with other organelles of the podocytes: nucleus, mitochondria, endoplasmic reticulum, lamellar apparatus, and also the plasma membrane. These observations are in agreement with data in the literature, indicating that microfilaments, together with microtubules, participate in information transmission between the plasma membrane and the internal

Murmansk Marine Biological Institute, Kola Branch, Academy of Sciences of the USSR, Dal'nye Zelentsy, Murmansk Region. Institute of Clinical Oncology, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Eksperimental'noi Biologii Meditsiny, Vol. 107, No. 5, pp. 633-637, May, 1989. Original article submitted March 15, 1988.

0007-4888/89/0005-0733$12.50

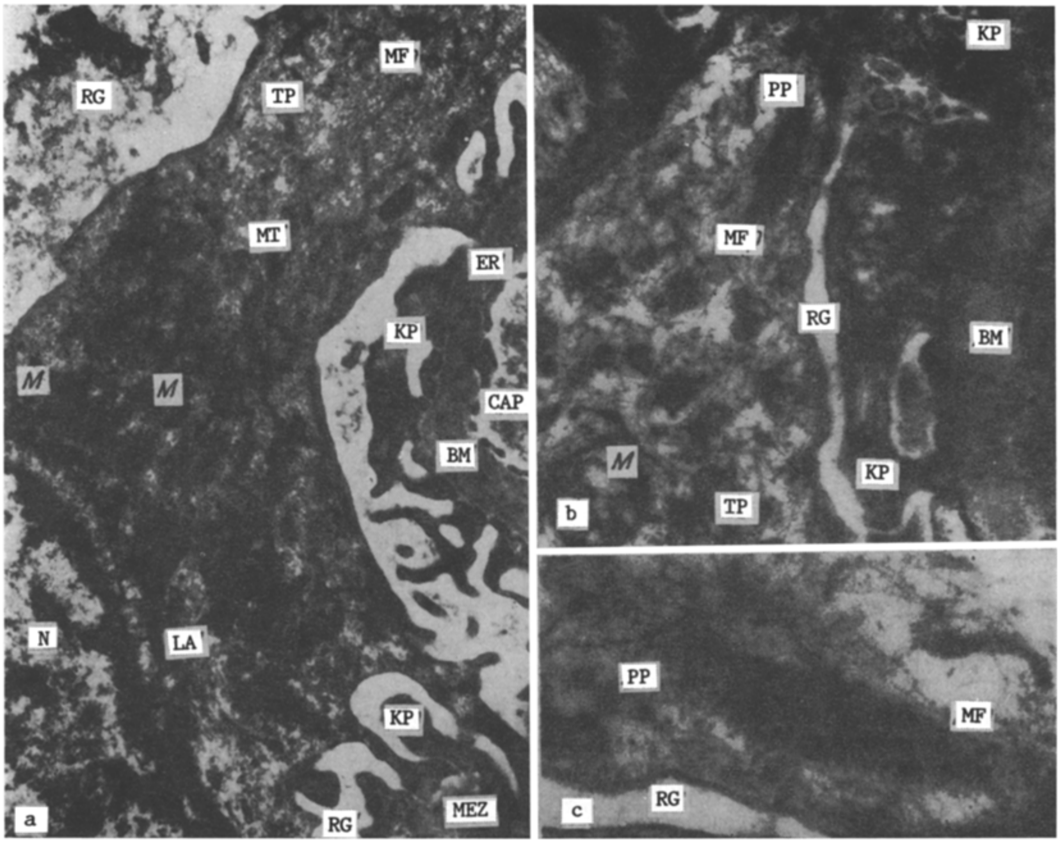

Fig. 1. System of microfilaments of renal podocytes of children aged 2, 4, and 6 years. Legend: RG) renal glomerulus; MF) microfilaments; TP) trabecular process; MT) microtubules; M) mitochondria; ER) endoplasmic reticulum; KP) knife-like process; CAP) capillary; BM) basement membrane; N) nucleus; LA) lamellar apparatus; PP) polysomes.

structures of the cell [13]. Fragments of filaments 17-19 nm thick, with transverse cross-striation, are observed extremely rarely in the podocytes. These fragments in podocytes of mammalian renal tubules, even if special methods of processing of the kidney tissue are used (the inert dehydration method), as Pease [12] points out, are seen only very palely. In his opinion, these fragments are myosin molecules, associated with tropomyosin. The morphological results are in agreement with biochemical data, indicating a low concentration of myosin in nonmuscular cells, in which it is found in the depolymerized form and accounts for only 1-1.5% of the total cell protein, whereas the content of actin polymers (microfilaments) reaches 10-15% [10]. Microtubules 24-25 nm thick are distributed in bundles in the podocytes or singly, mainly along the long axis of the cell bodies and their trabecular processes. The sites of "anchoring" of the microtubules to the plasmalemma of the podocytes and the multiple angles of their intersections and junctions of "end to side" type with microfilaments can be clearly distinguished. In recent years reports have been published to the effect that microtubules not only basically determine the geometric characteristics of the cell, but by their lengthening or shortening, they can also take part in cell movement [7]. Centrioles are well represented in the podocytes, where they are localized both in the perikaryon region and at the periphery of the cell, beneath the plasmalemma. Intermediate filaments were not found in the cytoskeleton of podocytes in the kidneys of children.

In the kidneys of a person aged 37 years two podocyte populations differing in the structure of their cytoskeleton were noted. The first type of cytoskeleton is identical in structure with that described above (Fig. 2a, b). The second type of cytoskeleton is characterized by a tendency toward replacement of the reticular structure of the microfilaments by a bundle type. It must be emphasized that bundles of intermediate filaments, with separate units 8-10 nm thick, are found in the cytoplasm of these podocytes (Fig. 3a). The intermediate filaments are the least known component of the cytoskeleton of eukaryote cells [11]. This applies most of all to the cytoskeleton of podocytes. The fact is that glomerular

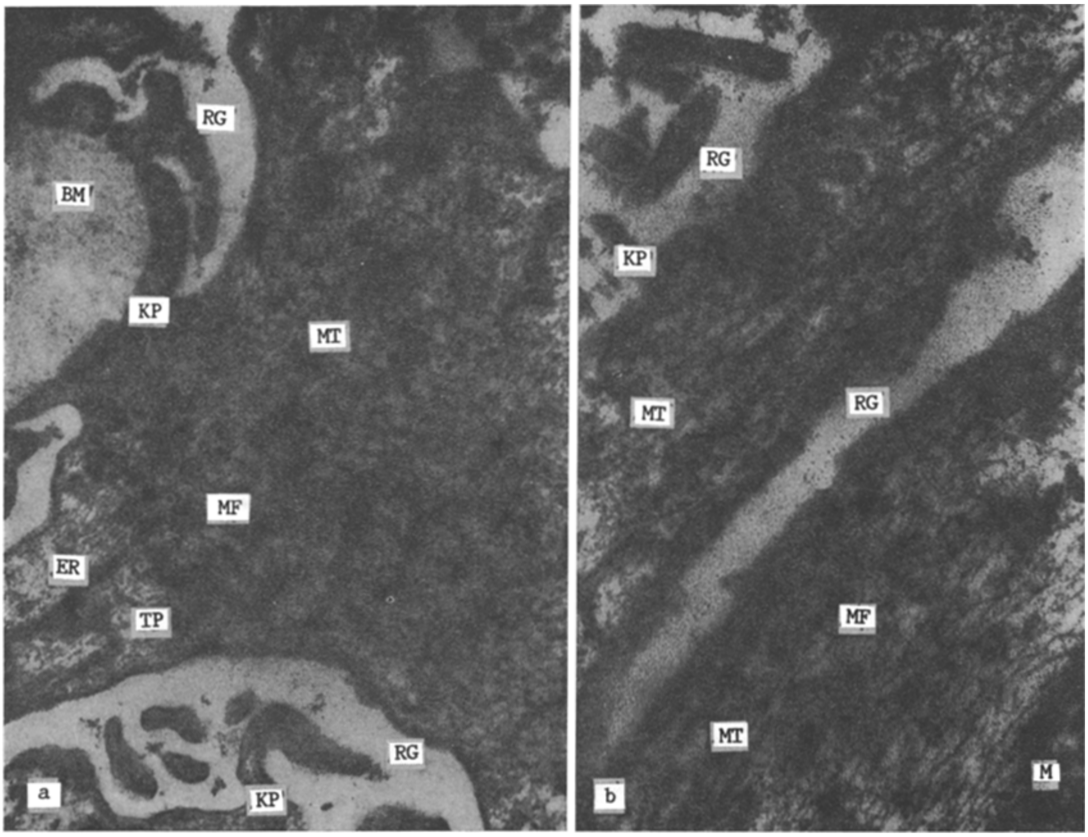

Fig. 2. Structure of podocytes in kidneys of a man aged 37 years.

podocytes, together with the epithelial cells of the lens, express intermediate filaments of only the vimentin type [14], which are not characteristic of them. This is a rare exception to the generally accepted classification of intermediate filaments, according to which cells of epithelial nature usually contain cytokeratin filaments. Microtubules and centrioles are single in this second variety of cytoskeleton.

In human kidneys at the age of 65 years, podocytes with the second type of structure of their cytoskeleton are predominant. Zones of transition of bundles of intermediate filaments from the bodies of the podocytes, where they are arranged in a circumnuclear manner, into trabecular and knife-like processes, and also their multiple junctions with the plasma membranes of the cell, can be clearly distinguished (Fig. 3b).

When these results are compared with those of an ultrastructural analysis of the cytoskeleton of podocytes in other mammals [2, 3, 8], the following state of affairs must be noted. Human kidneys contain two populations of podocytes with different types of structure of their cytoskeleton. The particular structural features of the podocyte cytoskeleton which have been found are observed equally well in both superficial, intercortical, and juxtaglomerular tubules. Type 1 is characterized by a branched, high-density network of microfilaments, a well developed system of microtubules, and single myofilaments. Intermediate filaments in this case are either completely absent or are so pale that they are "masked" by the other powerfully developed components of the cytoskeleton and they cannot be detected by electron microscopy. A similar situation with regard to detection of intermediate filaments in podocytes also occurred during the study of kidneys of other mammals, when it was concluded that special methods of investigation (immunofluorescence, immunoelectron microscopy, etc.) are needed for their discovery [3]. Podocytes with the cytoskeletal organization mentioned above evidently function actively only in the first half of human life, for podocytes with the type 2 cytoskeleton are more frequently observed in human kidneys at the age of 37 years. A bundle type of arrangement of the microfilaments, multiple bundles of intermediate filaments, and single microtubules are characteristic of this type of organization of the cytoskeleton. In human kidneys at the age of 65 years this type of cytoskeleton of the podocytes, with predominance of intermediate filaments, becomes the usual pattern. It is interesting to note that during a phylogenetic study of the vertebrate kidney, we observed the opposite

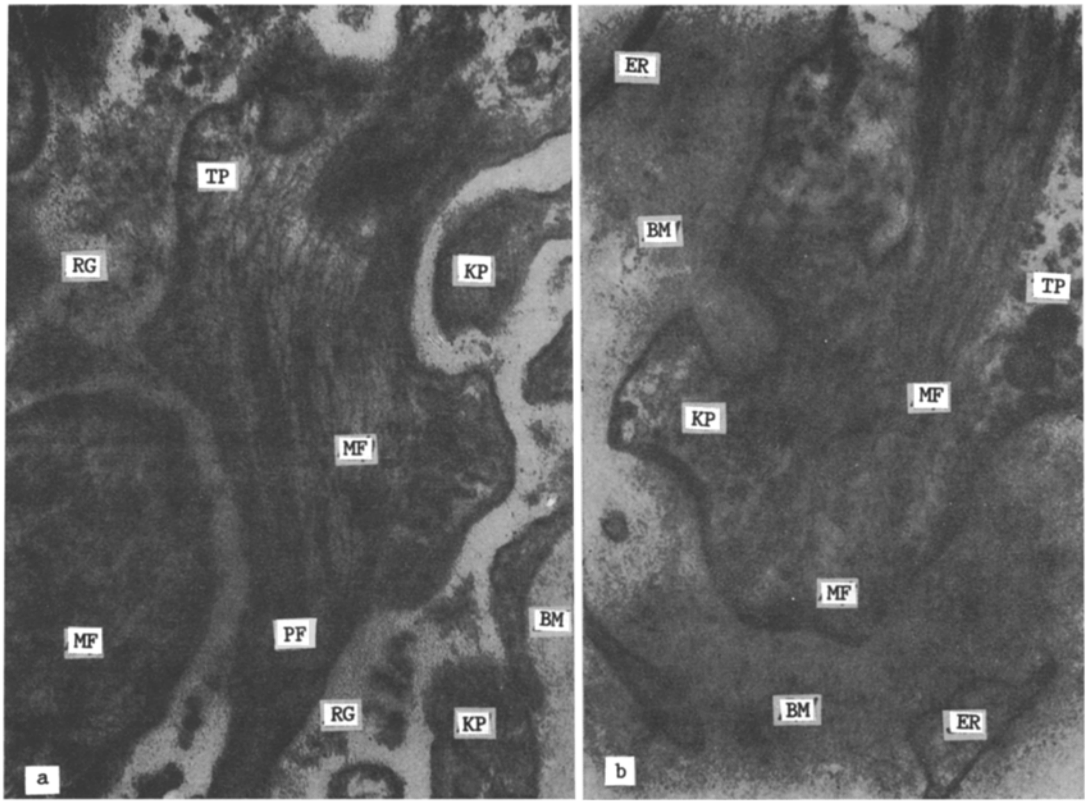

Fig. 3. Structure of podocytes in kidneys of a man aged 65 years.

tendency, namely gradual replacement of the powerfully developed system of intermediate filaments and microtubules, in the mammalian kidney [2, 3]. However, these results were obtained without consideration of the age factor. It can be concluded from this investigation that during individual development and aging, physiological changes determining the morphological reorganization of the podocyte cytoskeleton, which is accompanied by rapid development of the system of intermediate filaments and simultaneous involution of the systems of microtubules and microfilaments, may probably take place.

LITERATURE CITED

1. V. B. Zaitsev, Tsitologiya, 25, No. 3, 337 (1983).
2. V. B. Zaitsev and S. P. Gambaryan, Dokl. Akad. Nauk SSSR, 282, No. 4, 990 (1985).
3. V. B. Zaitsev and S. P. Gambaryan, Tsitologiya, 28, No. 4, 926 (1986).
4. Ya. L. Karaganov, A. A. Mironov, and V. A. Mironov, Usp. Sovrem. Biol., 84, No. 6, 5 (1983).
5. Yu. V. Natochin, Problems in Evolutionary Physiology of Water and Electrolyte Metabolism [in Russian], Leningrad (1984).
6. V. V. Serov, Clinical Nephrology [in Russian], Moscow (1983), pp. 9-33.
7. G. Albrecht-Buehler and A. Bushnell, J. Cell Biol., 95, 331 (1982).
8. P. M. Andrews and A. K. Coffey, Fed. Proc., 42, 3046 (1983).
9. W. W. Franke, K. Weber, M. Osborn, et al., Exp. Cell Res., 116, 429 (1978).
10. U. Groschel-Stewart, Int. Rev. Cytol., 65, 193 (1980).
11. E. Lazarides, Annu. Rev. Biochem., 51, 219 (1982).
12. D. C. Pease, J. Ultrastruct. Res., 23, 304 (1968).
13. J. E. Smolen, A. M. Korchak, and G. Weissman, Inflammation, 4, 145 (1980).
14. I. Virtanen, M. Miettinen, and V.-P. Lehto, Ontogenez, 15, No. 4, 422 (1984).