Expression of Neural Stem Cell Marker Nestin in the Kidney of Rats and Humans

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Nestin is present in podocytes of the renal corpuscle in rats and humans. Specific differences manifested in more intensive and widespread expression of nestin by endothelial cells of blood vessels in human kidney.

Key Words: *kidney; podocytes; blood vessels; nestin; mammals*

Nestin, an intermediate filament protein, plays a role in cytoskeleton formation in neural stem cells (NSC) [1]. Little is known about functional role of various proteins that form intermediate filaments in cells of various histogenetic types. Typing of biopsy specimens by the profile of intermediate filament proteins is an important method of modern histopathology [4]. During embryogenesis, nestin is expressed not only by NSC, but also by myogenic cells and other immature cells [5]. Published data show that nestin is present in highly differentiated cells, including podocytes of the renal corpuscle (RC) [5,7].

Species differences were revealed in the expression of intermediate filament proteins. It is interesting to compare the synthesis of nestin by renal cells in laboratory mammals and humans.

This work was designed to identify nestin-expressing cells in the kidney of rats and humans.

MATERIALS AND METHODS

Experiments were performed on kidney fragments from 12 male adult outbred rats (Rappolovo) and 5 adult humans (death not associated with diseases of the excretory system). The samples were obtained during elective biopsy and had no signs of autolysis. They were fixed with 2% formalin in 80% ethanol, 10% formalin, and zinc—ethanol—formaldehyde [2]. After fixation, the samples were dehydrated and embedded in paraffin. Serial sections (5 μ) were prepared on a Leica RM 2125RT rotation microtome. Nestin was detected immunohistochemically using mouse monoclonal antibodies (Rat-401, BD Pharmingen), rabbit polyclonal antibodies (Chemicon), secondary anti-mouse and anti-rabbit reagents (EnVision+), and DAB+ chromogen (Dako). After immunocytochemical study, some sections were stained with hemalum or Astra blue. A conjugate of streptavidin and FITC (Dako) was used for nestin detection by means of confocal microscopy. Cell nuclei were stained with a fluorescent dye propidium iodide. The preparations were examined under Zeiss LSM5 Pascal and Leica TCS SL confocal microscopes.

RESULTS

In rat renal cortex a strong reaction for nestin was observed only in RC cells (Fig. 1, *a*). These cells had large round or oval nuclei and were located outside the capillary loops. The shape of cells was estimated by means of confocal microscopy. Taking into account the structure of processes, these cells were identified as podocytes. Cell nuclei contained a considerable amount of euchromatin. Deep invaginations of the nuclear membrane were often revealed. In some samples, individual cells of the outer leaflet of the glomerular capsule and solitary dendritic cells of the interstitial connective tissue

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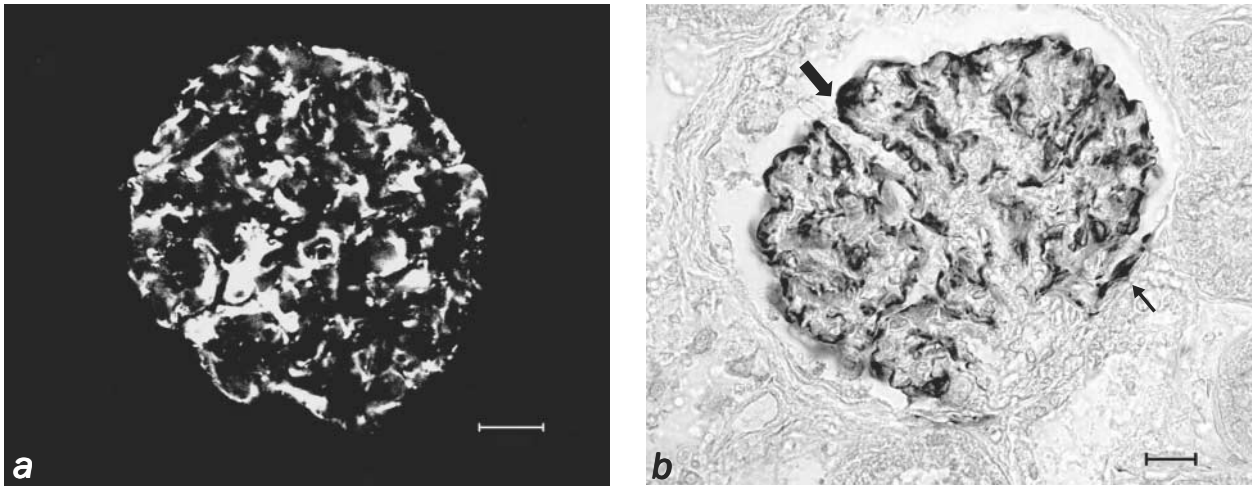

Fig. 1. Nestin-immunopositive cells in renal canaliculi of rats (*a*) and humans (*b*). Immunocytochemical reaction for nestin. *a*: confocal laser microscopy, fluorescence of the FITC channel, the fluorescence channel for propidium iodide is switched off; *b*: immunoperoxidase reaction with no staining (thin arrow, immunopositive squamous cell in the outer stripe of the glomerular capsule; thick arrow, dendritic cell body in the peripheral region of glomerular capillary loop). Scale 20 μ.

exhibited a weak reaction for nestin. In the renal medulla of rats, flat cells of thin-wall tubular structures (endothelium of blood vessels) were weakly positive for nestin. They were arranged in groups between tubules of the renal loop and collecting tubules.

In human kidney, strong reaction for nestin was typical of large dendritic cells in RC (Fig. 1, *b*). They were positioned along the outer surface of glomerular capillary loops (podocytes). A positive reaction for nestin was not detected in cells of the outer leaflet of the glomerular capsule. The exceptions were individual flat cells in some RC near the vascular pole (Fig. 1, *b*). Besides RC, endothelial cells of blood vessels (arteries and arterioles) in human renal cortex were characterized by positive reaction for nestin. In human renal medulla, endothelial cells of some arteries and small vessels in the intercanalicular space (probably, arterioles) were immunopositive for nestin.

Our results indicate that the kidney of rats and humans includes nestin-expressing cells. Nestin is extensively used as a marker of NSC. The reaction for nestin was particularly pronounced in RC podocytes.

It was hypothesized that only renal podocytes in mice and humans express nestin [7,8]. Our studies showed that nestin is expressed by vascular endothelial cells (mainly in arteries) not only in the cortex, but also in the medulla of human kidney. These data illustrate the existence of species-specific differences in nestin expression in the mammalian kidneys.

Podocytes have a specific function in the renal filtration barrier. At the same time, chemical composition of cytoskeletal proteins in highly differentiated cells (podocytes) is similar to that of the cytoskeleton in immature brain cells [1]. This fact is of interest. It should be emphasized that the presence of nestin is not the only marker, which illustrates close similarity of the cytoskeleton in podocytes and nerve cells. Intermediate filaments of podocytes contain glial fibrillary acidic protein [6], which serves as a specific marker of astrocytes [3]. The data attest to either common origin of these cells, or (more likely) to specific functional status of these structural elements of the filtration barrier and blood-brain barrier.

Our findings show that nestin is present in RC podocytes of rats and humans. Species differences in mammals are manifested in more intensive and widespread expression of nestin by endothelial cells of blood vessels in human kidney (compared to rat kidney). The prevalence of reaction for nestin in the endothelium of renal arterial vessels probably reflects functional differences between the endothelium of arteries and veins. The data on nestin expression by human kidney cells should be taken into account during immunophenotyping of renal tumors and metastatic dissemination of these tumors in the brain.

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