Three-dimensional microanatomy of the pericapillary mesangial tissues in the renal glomerulus: Comparative observations in four vertebrate classes

Hiromi TAKAHASHI-IWANAGA
Laboratory of Histology and Cytology, Graduate School of Medicine, Hokkaido University, Kita-15, Nishi-7, Sapporo 060-8638, Japan
(Received 31 July 2015; and accepted 15 August 2015)

ABSTRACT

The renal glomeruli in lower vertebrates display mesangium-like cells and matrices interposed between the capillary endothelium and the basement membrane, while those in mammals reportedly lack such interpositions except in pathological conditions. By combined scanning and transmission electron microscopic observations, the pericapillary mesangial tissues were comparatively analyzed in four vertebrate classes: mammals (rats and rabbits), reptiles (green iguanas), amphibians (bullfrogs), and teleosts (carps). The observations discriminated three types of pericapillary interposition. The first, acellular interpositions, occurred universally, with mammalians displaying rudimental ones. This tissue type corresponded with extracellular matrices held in subendothelial grooves which were supported by fine endothelial projections anchored to the basement membrane. In lower vertebrates these grooves constituted an anastomosed system of subendothelial channels that communicated with the mesangial region, to favor cleaning of the glomerular filter. The second, compound type was specific to reptiles and amphibians, affecting the entire capillary circumference in the latter. In this tissue type, fine mesangial processes—which accompanied considerable amounts of fibrillar matrices—were loosely associated with the endothelial bases, indicating their possible nature as a kind of myofibroblast. Occurrence of the third, cellular interpositions was confined to small incidental loci in mammalian and teleost glomeruli. This tissue type was mostly occupied by thick processes or main bodies of the mesangial cells that tightly interlocked their short marginal microvilli with corresponding indentations on the endothelial bases.

The mesangium is a connective tissue core that supports capillary loops of the renal glomeruli in all vertebrate classes. The glomerular capillaries are connected to the mesangium at a portion of their circumference, with the remaining portion being involved in the triple-layered barrier for glomerular filtration: the capillary endothelium, basement membrane, and the glomerular epithelium (17, 32, 33). All mammalian renal glomeruli—except those in developmental stages or pathological conditions—are characterized by the intimate apposition of the capillary endothelium to the basement membrane. On the other hand, lower vertebrate glomeruli are known to display pericapillary spaces filled with mesangium-like tissues between the endothelium and the basement membrane (6, 19, 20). Comparative analyses of the morphology of the pericapillary mesangial tissues should help in understanding the development of the mammalian renal glomeruli and their pathological alterations.

Previous transmission electron microscopy (TEM) observations in frogs demonstrated the frequent occurrence of fine cytoplasmic processes and cell bodies in the pericapillary spaces of renal glomeruli (19, 25, 30). In contrast, such cellular elements appear scanty in freshwater teleosts (3, 20). Scanning electron microscopy (SEM) after chemical or enzymatic treatment
removal of extracellular matrices enables extensive observation of cells associated with the capillaries, as demonstrated in mammalian renal glomeruli (13, 26). Concerning SEM of lower vertebrate glomeruli, however, we could find only one report in lungfish by Ojeda et al. (18), who demonstrated elongated cells or cell processes that bound most of the capillary circumference. More information is required concerning the entire shapes of the pericapillary cells and their topographical relationships with the accompanying capillaries in different vertebrate classes in order to clarify the above-mentioned interspecies differences.

Detailed TEM observations by Sakai and Kriz (23) demonstrated in rat renal glomeruli that the mesangial cells were mechanically linked with the basement membrane via fine bundles of extracellular fibrils running parallel to the pericapillary part of the latter. Makino and Ota (16) observed similar continuations of the mesangial matrix on the inner surface of the glomerular basement membrane by SEM after the removal of cellular elements. Little is known concerning homologues of these structures in lower vertebrate glomeruli.

To address the above-mentioned problems concerning the pericapillary mesangial extensions, we comparatively observed renal glomeruli in four vertebrate classes—mammals (rats and rabbits), reptiles (green iguanas), amphibians (bullfrogs) and freshwater teleosts (carps)—both by SEM after cell exposure with NaOH maceration (28, 29) and by TEM according to a conventional method.

MATERIALS AND METHODS

Animals. Wistar rats and New Zealand White rabbits were allowed free access to food and tap water. Green iguanas (Iguana iguana, Reptilia) and bullfrogs (Rana catesbeiana, Amphibia) were supplied by Aoki (Yokosuka, Japan) and by Sankyo Lab (Sapporo, Japan), respectively. Carp (Cyprinus carpio, Osteichthyes) were maintained in freshwater aquaria. Three adult individuals of each animal species were used in this study. Under anesthesia, the kidney of each animal was exposed and fixed by transcardial perfusion with a fixative containing 2.5% glutaraldehyde and 1.0% paraformaldehyde in 0.1 M cacodylate, pH 7.3, or by immersion in the same fixative.

SEM observation. The renal tissue pieces were immersed in the same fixative as used in the perfusion fixation for more than 6 h and processed with a modification of the NaOH maceration method (29). The specimens were placed in 6N NaOH for about 15 min at 60°C, then rinsed and mechanically disrupted with a glass pipette in a 0.02 M phosphate buffer (pH 7.3). The dispersed renal tissue was examined in a plastic Petri dish with a dissecting microscope. Renal corpuscles were collected with a micropipette and transferred into a round-bottom glass tube containing a fresh phosphate buffer (0.02 M, pH 7.3). The selected sediments of renal glomeruli were postfixed in 0.3% glutaraldehyde for 5 min, resuspended in a 0.1 M phosphate buffer (pH 7.3), and mounted on a glass coverslip coated with poly-L-lysine. The coverslip, mounted with glomeruli, was immersed in 1% tannic acid buffered with 0.1 M phosphate (pH 7.3) for 1 h, followed by 1% OsO4 buffered with phosphate (0.1 M, pH 7.2). The osmicated specimens were dehydrated through a graded series of ethanol, transferred to isoamyl acetate, and critical-point-dried with liquid CO2. The dried specimens were coated with osmium in a plasma osmium coater (Nippon Laser and Electronics Laboratory, Nagoya, Japan) and examined in a Hitachi H-4500 scanning electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 10 kV.

TEM observation. After the fixation, the renal tissue pieces were postfixed with 1% OsO4 in a 0.1 M phosphate buffer (pH 7.2) for 1.5 h and stained en bloc with uranyl acetate. The specimens were then dehydrated and embedded in Epon-812, sectioned at 90 nm, and examined in a Hitachi H-7100 transmission electron microscope after double staining with uranyl acetate and lead citrate.

RESULTS

SEM observation

NaOH maceration disintegrated the basement membrane and mesangial matrix in renal glomeruli and additionally induced epithelial detachment to expose the basal aspect of the capillary endothelium and free surfaces of the mesangial cells in all animal species examined (Figs. 1–4). The endothelium was fenestrated with numerous pores about 80 nm in diameter. The mesangial cells, via their processes, were attached to the basement membrane via fine bundles of extracellular fibrils running parallel to the pericapillary part of the latter. Makino and Ota (16) observed similar continuations of the mesangial matrix on the inner surface of the glomerular basement membrane by SEM after the removal of cellular elements. Little is known concerning homologues of these structures in lower vertebrate glomeruli.

To address the above-mentioned problems concerning the pericapillary mesangial extensions, we comparatively observed renal glomeruli in four vertebrate classes—mammals (rats and rabbits), reptiles (green iguanas), amphibians (bullfrogs) and freshwater teleosts (carps)—both by SEM after cell exposure with NaOH maceration (28, 29) and by TEM according to a conventional method.

MATERIALS AND METHODS

Animals. Wistar rats and New Zealand White rabbits were allowed free access to food and tap water. Green iguanas (Iguana iguana, Reptilia) and bullfrogs (Rana catesbeiana, Amphibia) were supplied by Aoki (Yokosuka, Japan) and by Sankyo Lab (Sapporo, Japan), respectively. Carp (Cyprinus carpio, Osteichthyes) were maintained in freshwater aquaria. Three adult individuals of each animal species were used in this study. Under anesthesia, the kidney of each animal was exposed and fixed by transcardial perfusion with a fixative containing 2.5% glutaraldehyde and 1.0% paraformaldehyde in 0.1 M cacodylate, pH 7.3, or by immersion in the same fixative.
Fig. 1  Scanning electron micrographs of mammalian renal glomeruli. a. A fenestrated endothelium (E) in a rat displays a smooth basal surface after removal of the basement membrane. Mesangial cells (M) are covered with short tapered microvilli. b. High magnification of a junction of a mesangium (M) and endothelium (E) in a rat. Short mesangial microvilli slide into corresponding grooves on the endothelial base (arrows). A fine subendothelial groove (arrowheads) extends into the mesangial region. c. A mesangial cell (M) in a rabbit extends a long pericapillary process (asterisks). Arrows indicate fine subendothelial grooves extending to the margin of the mesangial process. Bars 5 μm (a, c), 1 μm (b).
undulated with fine interdigitations between short tapered microvilli of the mesangial cells and corresponding indentations on the endothelial cells (Fig. 1b). Large mesangial processes, measuring 1–3 μm in width and more than 2 μm in length, were occasionally seen to extend beyond the primary contact zones into endothelial regions lying against the glomerular basement membrane. These pericapillary processes were also tightly interdigitated via their marginal microvilli with the endothelial cell bases. Some pericapillary mesangial processes in rabbits pursued circular or oblique courses about 10 μm long, with respect to the capillary axis (Fig. 1c). The endothelial bases lacking any mesangial contact were smooth both in rats and rabbits, except for fine subendothelial grooves, about 0.2 μm wide and no longer than 2.0 μm (Fig. 1b, c). The subendothelial grooves originated from small round openings in the cellular interdigitations at the mesangial junctions and pursued simple straight courses at right angles to the capillary axis. The grooves were bordered on both sides with fine ridges and perforated at the bottom with numerous fenestrae typical of the glomerular endothelium.

The cellular mesangial networks in green iguanas were frequently seen to insert their wing-like extensions into the pericapillary spaces. These mesangial extensions consisted of membranous cell processes loosely overlapping one another. These processes were bordered with microvilli, about 1.5 μm long, and extended tangentially with respect to the accompanying endothelial cylinder (Fig. 2). Only portions of the processes were attached to the endothelial bases, with the remaining free portions allowing communication between subendothelial spaces in the mesangial region and the pericapillary spaces. The basal surface of the endothelium in green iguanas was furrowed with numerous grooves, which mostly encircled the capillary circumference. The grooves ranged in width from 0.5 to 2 μm and converged at the margins of the pericapillary mesangial extensions. The endothelial bases displayed numerous microvilli and leaf-like microprojections on both sides of the grooves (Fig. 2b).

The bullfrog glomerular capillaries were thoroughly enveloped in an attenuate extension of the mesangial network, which ranged in thickness from 1 to 4 μm (Fig. 3a). The pericapillary mesangial tissue contained long ribbon-like cell processes, about 2 μm wide, which crossed and overlapped one another (Fig. 3b). The processes extended secondary and tertiary branches along the basal aspect of the capillary endothelium. Both the main processes and the branches were bordered with long microvilli, ranging in length from 1 to 2 μm. These microvilli were only loosely associated with the endothelial cell bases. The pericapillary mesangial processes additionally extended long microvilli solitarily or in small groups toward the overlying glomerular basement membrane. The endothelial cells frequently displayed short microvilli or small knob-like protrusions, no larger than 1 μm in size, along the entire extent of their basal surfaces.

The mesangial cells in carp appeared strikingly similar to those in mammals with regard to their tight connection to the endothelial bases via fine cellular interdigitations and occasional extensions of pericapillary cell processes (Fig. 4). One distinct feature in the carp glomeruli appeared in the presence of numerous subendothelial grooves that mostly encircled the capillary circumference (Fig. 4a). The grooves in carp, ranging in width from 0.2 to 1.5 μm, became thicker at every junction on their courses to the interdigitating boundaries of the mesangial junctions (Fig. 4b). Through their round orifices at the boundaries, these grooves were communicated with subendothelial spaces of the core mesangium. The subendothelial grooves were bordered on both sides with short microvilli and leaf-like microprojections. Larger grooves frequently displayed similar fine processes at the bottom.

**TEM observations**

In rats and rabbits the mesangial microvilli as well as the interlocked endothelial bases were closely associated with the glomerular basement membrane at the boundaries of the mesangial junctions (Fig. 5a). Thicker pericapillary processes of the mesangial cells were occasionally observed tightly interposed between the capillary endothelium and the basement membrane. Only small amounts of extracellular materials occurred about the pericapillary mesangial processes. The subendothelial grooves were represented by fine indentations, about 160 nm wide, on the basal surface of the cells (Fig. 5b). The grooves were filled with amorphous or fibrillar materials as dark as the basement membrane. The margins of the subendthelial grooves were tightly attached to the basement membrane, displaying a local enhancement in cytoplasmic density. In longitudinal sections, the aggregations of the extracellular matrix in the subendothelial grooves were seen to continue into those in the mesangial region through narrow gaps between the endothelium and the mesangial processes.

By TEM the wing-like pericapillary extensions of
the mesangium in green iguanas displayed considerable amounts of fibrillar matrices and fine profiles of cell processes dispersed among them (Fig. 5c). The capillary endothelium was lined by a layer of similar matrices ranging in thickness from 100–250 nm along the entire circumference. Short endothelial processes, varying in width from 0.5 to 2 μm, penetrated through the layer to connect to the glomerular basement membrane. The processes frequently displayed a local condensation of cytoplasm at the junctions (Fig. 5c).

The pericapillary extensions of the bullfrog mesangium contained large amounts of loose extracellular materials, amorphous or fibrillar in nature (Fig. 6a). Mesangial processes and their microvilli were sectioned apart from one another, exhibiting numerous fine profiles dispersed among the materials. Some microvilli were directly connected to the...
glomerular basement membrane, while others were linked with the structure via fibrillar materials of an intermediate electron density (Fig. 6b, c). In both cases the mesangial microvilli displayed a local condensation of superficial cytoplasm at the junctions. Portions of thick mesangial processes were closely associated with the basal aspect of the endothelium. However, an attenuate layer of amorphous matrix was always seen to interrupt the cell contact.

In cross sections of the carp renal glomeruli by TEM, thick mesangial processes as well as their main bodies were occasionally seen to affect the pericapillary spaces, with small amounts of amorphous materials accompanying the invading cells (Fig. 6d). Some portions of the glomerular endothelium were tightly attached to the basement mem-

brane, while others were locally separated from it by a generally 200 nm-thick deposition of flocculent extracellular material. Fine cell processes as dark as the endothelial cytoplasm occasionally occurred in the flocculent depostions.

DISCUSSION
Our combined SEM and TEM observations of renal glomeruli in four vertebrate classes distinguished three types of tissue interposition between the capillary endothelium and the basement membrane. The first, acellular type that invariably lacks mesangial cells but amply contains extracellular matrices, was observed rather universally among the animal species, though rudimental in mammals. The second,
Fig. 4 Scanning electron micrographs of teleost renal glomeruli. a. The capillary endothelium (E) and mesangium (M) are exposed after partial exfoliation of a podocyte layer (P). An erythrocyte protrudes through an artificial rupture in the capillary endothelium (asterisk). Note that an anastomosed system of subendothelial channels encircles the capillary wall (arrows). b. High magnification of a junction between the endothelium (E) and mesangium (M). Arrows indicate interdigitations between fine mesangial processes and corresponding indentations on the endothelial cell base. Arrowheads, subendothelial grooves. c. A pericapillary mesangial process (asterisk) is tightly attached to a basal surface of the endothelium (E) via fine cellular interdigitations (arrows). Arrowheads, subendothelial grooves; M, mesangium. Bars 10 μm (a), 1 μm (b, c).
compound type that comprises numerous fine mesangial processes and considerable amounts of fibrillar matrices was specific to reptiles and amphibians, as far as we examined. The third, cellular type that is mostly occupied by cytoplasmic processes or main bodies of the mesangial cells was seen to occur at small incidental loci in the mammalian and teleost renal glomeruli. Comparative TEM analyses in seven vertebrate classes previously reported the renal glomeruli to be affected by decreasing amounts of pericapillary mesangial extensions according to evolutionary development (6). In our observations, however, the mammalian glomeruli rather abruptly appeared to lose the extensive pericapillary interpositions as observed in lower vertebrates (reptiles, amphibians and teleosts) due to the acute diminishment of the acellular type. By contrast, the occurrence of the other two types, compound and cellular, varied among animal species independent of evolutionary stage.
The pericapillary spaces that had contained the acellular interpositions were seen to accompany fine endothelial protrusions anchored to the glomerular basement membrane both in mammals and in lower vertebrates. Similar structures have been previously demonstrated by TEM in reptiles (Figs. 9 and 10a in ref. 6, Fig. 3 in ref. 10, Fig. 8 in ref. 21), amphibians (24, 25), and teleosts (4). One distinct feature of the subendothelial indentations observed by SEM is that they correspond to elongated grooves pouring into the mesangium after their circular coursing on the capillary wall. Especially in lower vertebrates, these grooves mostly encircled the capillary circumference and frequently joined with each other to constitute an anastomosed system of subendothelial channels communicating with the mesangial region. Previous TEM experiments with intravascular tracer particles have characterized the glomerular basement membrane as a principal barrier for plasma macromolecules in the urinary filtration (5, 21). The sub-
endothelial channels appear advantageous for the drainage of the filtration residues along the inner aspect of the basement membrane into the mesangial region, where the residues are supposedly endocytosed by mesangial cells or recycled into the lumen of the efferent capillary loops (8, 14). The extreme diminishment of such a drainage system in the mammalian glomeruli may require alternative mechanisms for cleaning of the filter.

By their detailed TEM observations of rat renal glomeruli, Sakai and Kriz (23) demonstrated fibrillar links between the mesangial processes and the pericapillary basement membrane and regarded the structures as mechanically supporting the capillary wall. Makino and Ota (16) confirmed the presence of such fine appendages on the inner side of the basement membrane by SEM observation after the chemical removal of cellular elements. These structures correspond closely to the fine acellular interpositions in the mammalian glomeruli as observed in this study with regard to their circular courses to the capillary axis and their communication with the mesangium. Previous TEM studies have shown mammalian renal glomeruli to display large amounts of pericapillary deposits of extracellular materials, including rudimental basal lamina, in an early developmental stage (1, 22). These findings suggest that the fibrillar supports of the capillary wall in the mature glomeruli correspond to remnants of a subendothelial channel system for cleaning of the glomerular filter at an immature stage.

In contrast to the rather consistent occurrence of the acellular type interpositions in lower vertebrate glomeruli, the compound type ones markedly varied in the degree of their pericapillary invasion: the capillary circumference was only partially held by the tissue in green iguanas, and was completely encircled in bullfrogs. Previous TEM observations suggest the existence of such variations among reptilian species; a pericapillary tissue with dispersed cell processes was reported to surround most of the capillary circumference in the lizard Podarcis taurica (10), while such mesangial tissue was observed confined to the axial region of the garter snake glomeruli (21). In the amphibian Rana esculenta, a pericapillary mesangial matrix and mesangial cell extensions were reported to increase in amount during hibernation (9). Reptiles and amphibians are known to respond to environmental perturbations, such as dehydration, by transient reductions in the glomerular filtration rate (31). These findings suggest the involvement of the compound type interpositions in tissue plasticity for renal acclimation to the habitat.

More extensive analyses will be required concerning inter- and intraspecies variations in the morphology of this type of pericapillary interposition.

Mesangial cells supplying the pericapillary processes in reptiles and amphibians differ from mammalian mesangial cells with regard to their absence of interdigitations with the endothelial bases (13, 26, 27), as well as to the presence of a certain amount of fibrils in their accompanying matrix. Nevertheless, the former cells resemble the latter in that they mechanically support the tissue with their microvilli, or “spinous processes (8)” anchored to the inner aspect of the glomerular basement membrane (23). Accumulating data have characterized the mammalian mesangial cells as specialized vascular pericytes essential for vessel stability (2), as well as pointing to the ability of these cells to transform into myofibroblasts linked with severe fibrosis of the renal glomeruli (7, 12). Dissociation of the pericytes from the capillary has been described as one of the earliest events in fibrogenic changes in the pericyte phenotype (15). The above-mentioned features of the reptilian and the amphibian mesangial cells may indicate a certain degree of their phenotypic shift to a myofibroblast. The regulatory mechanisms for the expression of the cell features require elucidation.

The pericapillary interpositions of the cellular type in the mammalian and the teleost glomeruli are regarded as normal extensions of the mesangium in the axial region of the glomerulus, based on their anatomical continuity and their common morphological features. The pericapillary mesangial processes as well as those in the axial region were seen to tightly associate their marginal microvilli with the endothelial bases and to accompany subendothelial grooves that can contain supporting elements for the capillary wall. A growing body of evidence indicates an essential role for the mesangial cells to support the capillary wall mechanically in development and the maintenance of the renal glomerular tufts (11, 15, 23). The spatially-confined occurrence and the variable degrees of extension of the pericapillary mesangial processes may imply an equalization of the wall tension of the glomerular capillaries for the prevention of local ballooning or microaneurysms. Comparative analyses of the three-dimensional mesangial structure in different animal species will advance our understanding of the development of the renal glomeruli as well as the mechanisms for the progress and recovery of renal diseases.
REFERENCES


