Desmosomes as markers for the proliferating parietal epithelial cells in collapsing glomerulonephritis. A case report

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Abstract
Two variants of focal segmental glomerulosclerosis are known to present epithelial hypercellularity in the Bowman’s space, namely the collapsing and the cellular types. This epithelial cell proliferation may get features of either pseudocrescent or tubular profiles. Our case of collapsing focal segmental glomerulosclerosis has been ultrastructurally investigated concerning the proliferating epithelial cell type: parietal versus visceral. Based on the cellular organelles, especially on the ubiquitous presence of desmosomes, the authors are endorsing, with ultrastructural arguments, the opinion favoring the parietal epithelial cells (PEC) as the proliferating cell type. It is also taken into consideration the eventual change of PECs phenotype in contact with the glomerular tuft components like the glomerular basement membrane.

Keywords: glomerulosclerosis, parietal epithelial hypercellularity, desmosomes.

Introduction
Focal segmental glomerulosclerosis (FSGS) has been defined as a syndrome manifesting proteinuria, usually of nephrotic range, associated with both focal, segmental glomerular sclerosis and podocytopathy. Besides, hyaline insudation and foam cells are common, but dense deposits of immune complexes are always lacking. Alterations of the podocyte cytoarchitecture constitute the major ultrastructural findings [1]. Early in the disease process, the pattern of glomerular sclerosis is focal, touching a subset of glomeruli, and segmental, involving only a portion of the glomerular tuft. As the disease progresses, it evolves into a more diffuse and global sclerosis [2]. Even in the early 1980s, pathologists have drawn the attention to the “cellular lesion” involving extracapillary and endocapillary hypercellularity [3]. The Columbia FSGS Classification distinguishes five variants of the disease: collapsing, glomerular tip lesion, cellular, perihilar and not otherwise specified. Two of these five variants (collapsing and cellular) may share the feature of extracapillary hypercellularity owing to podocyte hyperplasia, as an almost general opinion. Podocytes may appear swollen and crowded, sometimes forming “pseudocrescents”. These pseudocrescents can be distinguished from true crescents by their lack of attachment to Bowman’s capsule or continuity with the parietal epithelial cells [4]. This interpretation of the extracapillary hypercellularity was largely endorsed by many pathologists [2, 4–6].

In 1996, in the book “La biopsie rénale”, edited by D. Droz and B. Lantz, the authors conclude that in one glomerulus, in between the capsulo-glomerular adherences, the urinary space becomes closed. When several adherences realize such spaces they are completely surrounded by epithelial cells which are some parietal and some seem to be podocytes [7].

During the last decade, several papers by a group of investigators lead by Bart Smeets [8–10] brought strong immunohistochemical arguments on experimental models, showing that PECs constitute the main part of cells composing the extracapillary proliferative lesions in both crescentic and collapsing glomerulopathies FSGS including. In a parallel study on human biopsies, the same authors also found that proliferative lesions in patients with crescentic glomerulonephritis and collapsing glomerulonephritis predominantly contained PECs [11]. The hyperplasic cells were identified by their expression of the renal progenitors’ markers CD113 and CD24, similar to the epithelial cells on Bowman’s capsule [12, 13].

Our ultrastructural investigation on a case of FSGS collapsing variant showed some additional ultrastructural arguments from human biopsy samples, supporting the massive parietal epithelial cells contribution in this process of epithelial hypertrophy and hyperplasia.

Patient and Methods
A 61-year-old female patient was admitted in the hospital for oliguria, proteinuria and edema of about two months duration. She was previously diagnosed (at the age 44) with breast cancer and underwent surgery, radiotherapy and chemotherapy. Ten years later bone metastasis were discovered and recently liver metastasis also.

In the past year, she was treated with Bevacizumab, Gemcitabine, Docetaxel (discontinued because of proteinuria) and intermittently with Pamidronate.

Her last laboratory studies showed proteinuria of 4.11 g/24 h with normal renal function. At the admission...
in our hospital, on physical examination: alopecia, right palpebral ptosis, exophthalmia, mydriasis, pitting edema, left upper limb lymphedema, blood pressure of 160/80 mmHg, ascitis, and hepatomegaly were present.

Investigations showed: hypoalbuminemia (2.93 g/dL), inflammation (C-reactive protein 20 mg/L), a serum creatinine of 1.23 mg/dL, glomerular filtration rate (GFR-EPI) +47 mL/min./1.73 m². HIV test was negative.

On urinalysis there was proteinuria 4+, 210 RBCs/high-power field, leukocyturia, hyaline and granular casts. There were 4.11 g/24h proteins.

The case was interpreted as acute nephrotic syndrome and renal biopsy was recommended and performed with a GBL 16/15 guillotine needle. One kidney fragment of 20 mm has been harvested and parted for immuno-fluorescence and for electron microscopy. Thus, the frozen sections have been stained with FITC conjugated antibodies as usual for diagnosis.

The 1-mm³ fragments for electron microscopy have been fixed in 4% buffered (sodium cacodylate) glutaraldehyde for four hours, then post-fixed in 1% buffered osmium tetroxide, and after dehydration the Epon embedding followed.

The light microscopy has been performed on Toluidine blue stained thick sections and the appropriate glomeruli have been oriented for ultrathin sections of 60 nm. These sections have been double stained and finally examined with a JEM 1011 transmission electron microscope (JEOL, Tokyo, Japan).

Results

The immunostaining of the frozen sections was moderately positive only for C3 and IgM. The light microscopy showed lobular and segmental solidifications in the majority of glomeruli associated with capillary adhesions. Individual capillaries or entire lobules were hyalinised and some small lipid vacuoles were also seen (Figure 1). In some glomeruli, the capillaries have been collapsed with no remaining lumina. A few red blood cells have been found in tubules and in the urinary space. Some interstitial foam cells were seen here and there.

In a few glomeruli, we observed an epithelial hypercellularity around the collapsed glomeruli. This extra-capillary proliferation seemed to define tubular profiles in some areas (Figure 2). A discreet periglomerular inflammation was also noted. The diagnosis has been concluded in favor of a collapsing variant of FSGS. An admissible cause of the disease has been the bisphosphonates treatment [8].

The electron microscopy was used to investigate the epithelial hypertrophy and hyperplasia. Thus, we could find details concerning the cell type of the extracapillary hypercellularity. The PECs were flat or a little bit rounded. In several points, where adherences were detected in light microscopy, we found some epithelial cell clusters developed in a polypous style. We could compare these proliferations with mushrooms, having a stalk with a central axis of basement membrane material and collagen fibers in direct continuity with the Bowman’s capsule (Figures 3 and 4). These proliferating cell complexes showed also further ramifications (Figure 6). All of the participating cells were epithelial, parietal type, with few short microvilli and most important interconnected with desmosomes. On the opposite side, by the collapsed lobules, these proliferating epithelial structures were in direct contact with the hyaline masses (Figures 4 and 6). At higher magnification, we have seen that all these epithelial cells in contact with the collapsed lobule have been of the same parietal type and interconnected with desmosomes (Figures 5 and 7). In other words, all the epithelial cells composing these extracapillary proliferations possessed desmosomes as the only tight junction interconnecting them. We also found few PECs in mitosis.

Discussion

Most authors are currently interpreting the extracapillary hypercellularity as podocyte hyperplasia [2, 4–6, 10]. This assertion is, in a way, in contradiction with the more recent concept that podocytes are highly differentiated, post mitotic terminally cells, deprived of cell division capacity and of self repair, in case of detachment, apoptosis or necrosis [14, 15].

Figure 1 – Two glomeruli showing lobular sclerosis, hyalinised capillaries and several adhesions to Bowman’s capsule. The glomerulus on the right shows hyalinised lobules and a few lipid vacuoles (arrow). Toluidine blue staining. Scale bar = 500 px.

Figure 2 – Collapsing type glomerulosclerosis. The epithelial proliferation in the Bowman’s space contains several tubular profiles. In between these profiles, one can see adhering points of the tuft to Bowman’s capsule (arrows). Toluidine blue staining. Scale bar = 500 px.
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Figure 3 – Adhesion between the Bowman’s capsule (BC) and a segmental sclerotic lobule (SG). The proliferating epithelial bridge is centered by a collagenous extracellular matrix axis (arrow). Urinary space (US) fragmented in smaller areas as tubular profiles. Electron microscopy of a double stained thin section.

Figure 4 – Glomerular adhesion between Bowman’s capsule (BC) and solidified lobule (SL). Collagenous axis (arrow). Urinary space (US). Parietal epithelial cells (PEp). The inset rectangle is enlarged in Figure 5.

Figure 5 – Multiplication of the Figure 4 inset shows the proliferating epithelial cells interconnected by several desmosomes (arrows). Sclerotic glomerular capillary with hyalinosis (H).

Figure 6 – Additional capsulo-glomerular adherence, and proliferating parietal epithelial cells (PEp). Bowman’s capsule (BC), Urinary space (US). Segmental sclerosis with hyaline deposit (H). The inset rectangle is enlarged in Figure 7.

Figure 7 – Enlarged rectangular area from Figure 6 showing desmosomes (arrows) linking the neighboring epithelial cells in contact with a sclero-hyalinised lobule (SL). Urinary space (US).

In the past decade, new insights concerning the podocytes derived from kidney diseases and experimental models [16–18]. With only one exception, podocytes do not proliferate in the glomerular diseases [15]. The exception is the nephropathy associated to the HIV infection [19], in which the injured cells dedifferentiate to an immature state, thus reviving the cell cycle [20].

Taking into consideration the fact that parietal epithelial cells possess desmosomes as intercellular tight junctions while podocytes do not, we appreciate the presence of desmosomes as a cell marker to differentiate between these two types of epithelial cells lining the outer and the inner sides of Bowman’s space. In our case, the extracapillary proliferation contained exclusively epithelial cells connected in between by desmosomes. The epithelial gatherings shaped adhering bridges between the glomerular capillary tuft and the Bowman’s capsule that contained strips of collagenous extracellular matrix as support. We consider this feature a strong argument for the essential
contribution of PECs to the extracapillary hypertrophic–hyperplastic process. This parietal epithelial cell composition can also explain the tendency of tubular profiles formation.

The mouse model (Thy-1.1 transgenic mice) used by Smeets et al. (2004) developed structural lesions closely resembling the collapsing variant of human FSGS [8]. The phenotypic analysis of Ki67-positive proliferating epithelial cells showed their localization in high percent in the Bowman’s space, adjacent to adhesions. By double immunofluorescence, the authors never observed co-localization of Ki67 and the podocyte specific Thy-1.1 antigen, an argument for the lack of proliferating podocytes. In addition, their evaluation of the scar extracellular matrix composition, with antibodies against heparan sulfates and collagen IV α2 and α4, indicated that this matrix derived from PECs. Their hypothetical sequence of events leading to cellular FSGS is very similar with our ultrastructural features on human samples.

Thus, our results strongly endorse the opinion that PECs play the main role in the epithelial proliferation in primary collapsing FSGS.

There is also the possibility that some of the proliferating PECs be so-called stem or progenitor cells with capacity to differentiate into podocytes. Such an evolution could be considered in a later step. Research using a transgenic animals’ model indicated the presence in the parietal epithelium of podocyte progenitor cells that migrate over the basement membrane of the Bowman’s capsule to ensure a constant re-supply of podocytes [11, 14, 21]. Our results are in agreement with this opinion and give support to a migrating process of PECs toward the glomerular tuft. This hypothesis may become true, providing that the differentiation into podocytes should take place subsequently, after the contact of PECs with the glomerular basement membrane of the collapsing tuft.

Conclusions

Our opinion, based on the presence of desmosomes, is that the extracapillary proliferation during FSGS cellular variant is built up, at least in the early phase, almost exclusively on the expense of PECs that may also contain some putative renal progenitor cells. Thus, our results are strongly sustaining with ultrastructural features from human samples, the important role of PECs in the collapsing glomerulosclerosis.

References


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