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Nanostructural features of diabetic podocytopathy

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Abstract

The earliest glomerular lesion during the diabetic nephropathy is considered by many authors to be the so-called podocytopathy. Microalbuminuria is an early clinical marker of diabetic nephropathy that results from damages of the glomerular filtration barrier at the level of the highly differentiated podocytes. Thus, the diabetic podocytopathy includes cellular hypertrophy, foot process effacement, detachment from glomerular basement membrane (GBM), and apoptosis. The present paper is reviewing all these features and some additional ultrastructural transformations concerning the podocytes involved in the diabetic kidney disease.

Keywords: diabetes mellitus, podocytopathy, podocytes, foot processes.

Introduction

Podocytes are highly specialized cells with multiple structural and metabolic functions, among which the filtration barrier, the normal structure of the glomerular tuft, controlling the intracapillary hydrostatic pressure, and remodeling the GBM.

Podocytes have a cell body with the nucleus, primary processes, and foot processes placed on the GBM, and interconnected by slit diaphragms. The complex structure of podocytes can be altered by many factors: abnormalities in transcription, altered mitochondria, aberrant lysosomal activity or proteins residing in the luminal or abluminal surface of the cell membrane, either facing the GBM or participating in the slit diaphragm complex, or in the actin-based cytoskeleton. Abnormal podocyte function can be determined by intrinsic damages, or by extrinsic factors. As a consequence the podocytes may react in different ways: (1) foot processes effacement due to a modified phenotype; (2) apoptosis; (3) development arrest as immature precursors associated with mild proliferative activity and diffuse mesangial sclerosis; (4) dedifferentiated adult podocytes followed by marked proliferation and collapsing glomerulopathy [1].

Nephrin and podocin, the two proteins that comprise the slit diaphragm (SD) are essential for a good function of the filtration barrier. The maintenance of foot process structure is dependent on SD proteins [2]. In diabetes mellitus, abnormalities of the SD complex result in more then one morphologic pattern of injury meaning either focal glomerular sclerosis or diffuse mesangial sclerosis [3].

The preservation of appropriate podocyte adherence to the underlying GBM is critical for maintenance of normal cell function. A hallmark of diseases of the glomerular filter is podocyte detachment, resulting in the detection of podocytes in urine [4]. The interaction podocyte–GBM is modulated by both intrinsic podocyte proteins and extracellular matrix constituents of GBM. Integrin and dystroglycan heterodimers serve as a bridge between matrix proteins and the podocyte contractile apparatus.

Every type of podocytopathy has its own characteristics. The diabetic podocytopathy feats the first and the third pathways of podocyte injury with injured slit diaphragms, increased mesangial matrix and diffuse mesangial sclerosis. Thus, the structural damages of podocytes are consistent with microalbuminuria and proteinuria as the earliest clinical markers of diabetic nephropathy [5].

The form and function of glomerular filtration sieve depends primarily on the steady state of the intricate podocyte network and its slit diaphragm. Injury of this network is the key event in the diabetic podocytopathy. While glomerular hypertrophy, mesangial matrix expansion, and GBM thickening are classical hallmarks of diabetic glomerular lesions, the onset of albuminuria is most closely associated with podocytopathies including foot processes effacement, podocyte hypertrophy, detachment, apoptosis, and perhaps epithelio-mesenchymal transition [6]. During this process, epithelial cells lose intercellular contacts and undergo a reorganization of the actin cytoskeleton. Thus, podocytes revert to an immature, undifferentiated phenotype, the TGF-β cytokine being a potent inducer of trans-differentiation [7]. Consequently, the new phenotype of podocytes during diabetic kidney disease results in detachment from GBM and podocytopenia [8, 9].

The present study has investigated the ultrastructure

of podocytes and their cytoplasmic compartments in diabetic experimental animals.

Materials and Methods

For the ultrastructural investigation, we have used two lines of stabilized obesity-prone mice, which spontaneously develop diabetes mellitus. The first line was labeled BKS.Cg-m+/+Leprdb/J, and the second dTq, provided by "Ioan Cantacuzino" Institute.

Six mice have been sacrificed during the first six months, one each month, with general anesthesia according to the rules established by "Victor Babeş" Institute Ethics Board. This timing was intended for finding out the moment of glomerular lesions occurrence. Later on the mice have been sacrificed by two every six months.

Kidney samples have been harvested after aortic perfusion with 1.5% glutaraldehyde for 10 minutes. After this perfusion, kidneys became pale and though. Small samples of about 1 mm^3 have been cut with a sharp razor blade and immersed in 4% buffered glutaraldehyde. Later on, the samples were washed overnight in sodium cacodylate buffer at a 7.3 pH. After post-fixation with 1% buffered osmic acid one hour, the small blocks were dehydrated in graded alcohols and embedded in Epon.

One micron thick sections stained with Toluidine Blue were examined in light microscopy, and the glomerular lesions were studied with a special focus on podocytes. Later, the most affected glomeruli were targeted for ultrastructural analysis. Thin sections of 80 nm were double stained, and the study was performed with a JEOL JEM 1011 electron microscope at 80 kV.

Results

The first ultrastructural lesions have been noticed after 12 months since mice became diabetic, and were the GBM discontinuous thickening, and the podocyte hypertrophy. The podocyte injuries have been associated with those of GBM. GBM presented discontinuous thickenings along the glomerular capillaries, and were constant throughout the experiment.

The early podocyte transformations were hypertrophy and swelling. Hypertrophy resided in the increased volume and number of organelles involved in protein synthesis. Thus, rough endoplasmic reticulum and free ribosomes were much developed (Figure 1), and several Golgi apparatuses occurred in the cytoplasm of podocytes (Figure 2).

Swelling of podocytes also occurred as a later aspect of cell injury. The cytoplasm area looked like ballooned, full of cellular matrix but organelles were sparse (Figure 3).

Another feature often observed was cytoplasmic lipid inclusions. These vacuoles were set up of several small lipid droplets packed together and surrounded by an endomembrane (Figure 4).

Another stage of cell injury was the podocyte fragmentation. Thus, the podocyte cell body lost contact with the foot processes because the primary processes were more or less disintegrated. The urinary space contained small and bigger cytoplasmic fragments (Figure 5).

Apart from the flattened or effaced foot processes and cytoplasmic fragments, the glomerular extracapillary space contained also numerous microvilli (Figure 6).

An additional aspect of podocyte injury occurred later, after 18 months, as an atrophic feature of these epithelial cells. In spite of the foot processes presence, the primary processes became more and more slender, the cytoplasmic organelles were also small and sparse and acquired astonishing lengths (Figure 7). These much flattened podocyte processes have dilatations from place to place (Figure 8).

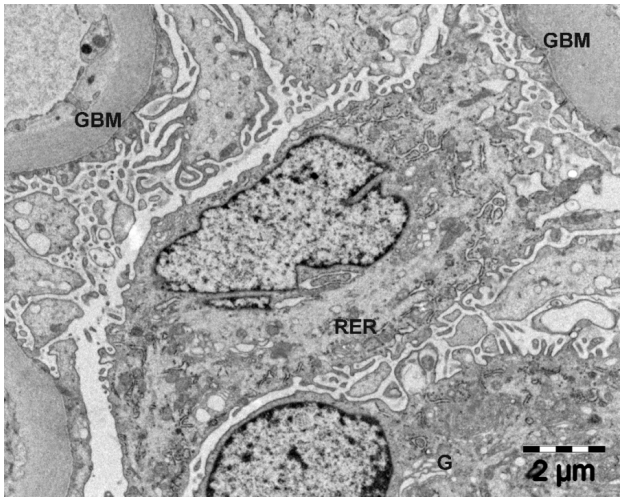

Figure 1 – ***Hypertrophic podocyte, 12 months after hyperglycemia started. Large cytoplasmic body containing developed rough endoplasmic reticulum (RER) and enlarged Golgi apparatus (G). Thickened glomerular basement membrane (GBM). Double stained thin Epon section.***

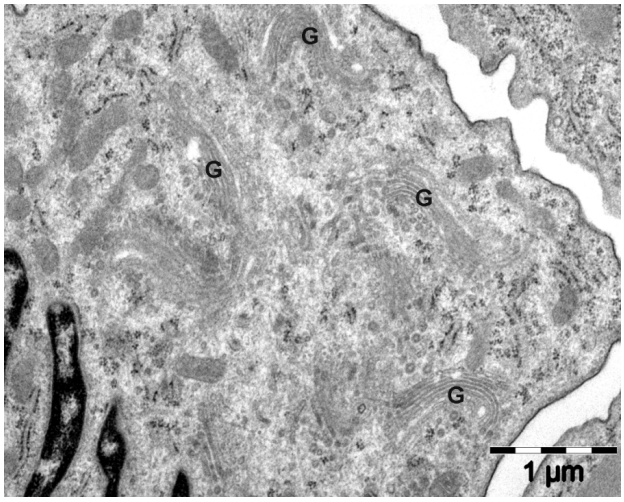

Figure 2 – ***Hypertrophic podocyte showing four Golgi apparatuses (G). Double stained section.***

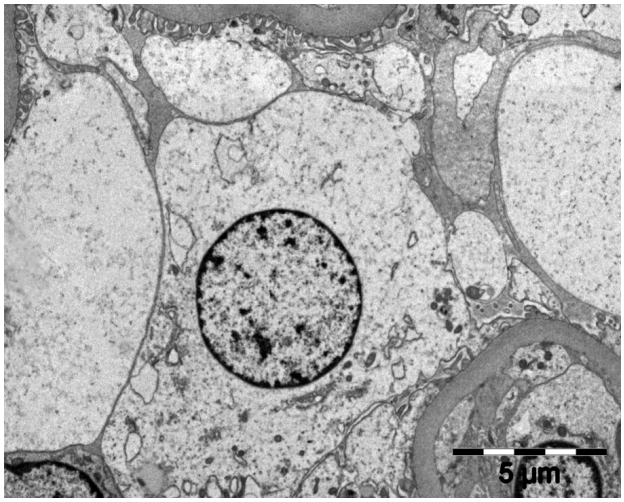

Figure 3 – ***Podocyte swelling. Ballooned cytoplasm with almost empty cytoplasmic matrix. Double staining.***

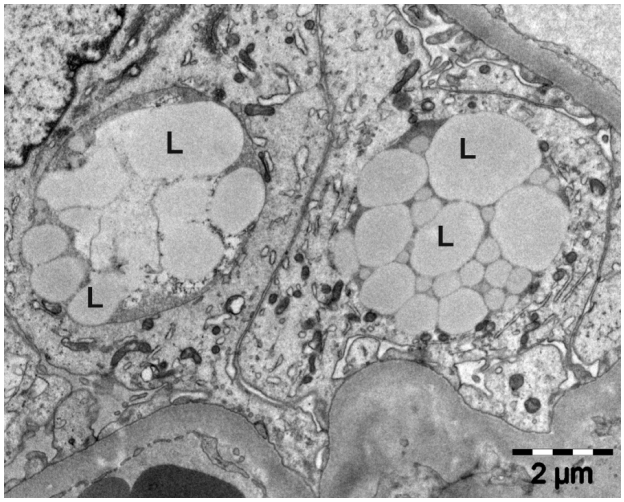

Figure 4 – ***Podocyte with cytoplasmic lipid inclusions. Lipid droplets (L) surrounded by an endomembrane.***

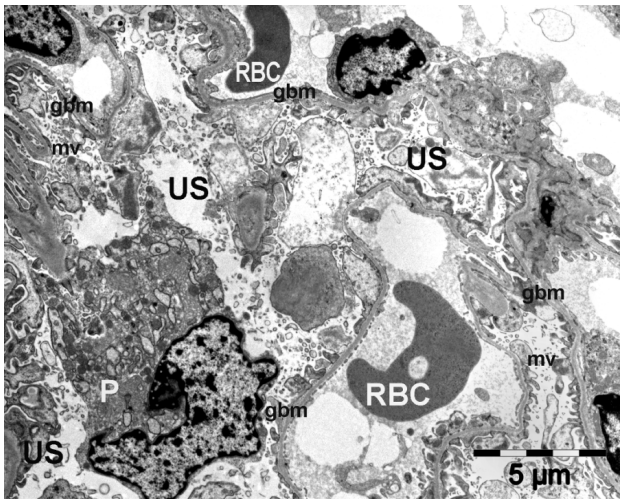

Figure 5 – ***Fragmented cytoplasmic processes of a podocyte (P). Urinary space (US) containing cytoplasmic fragments and microvilli (mv). Red blood cells (RBC). Capillary basement membrane (gbm).***

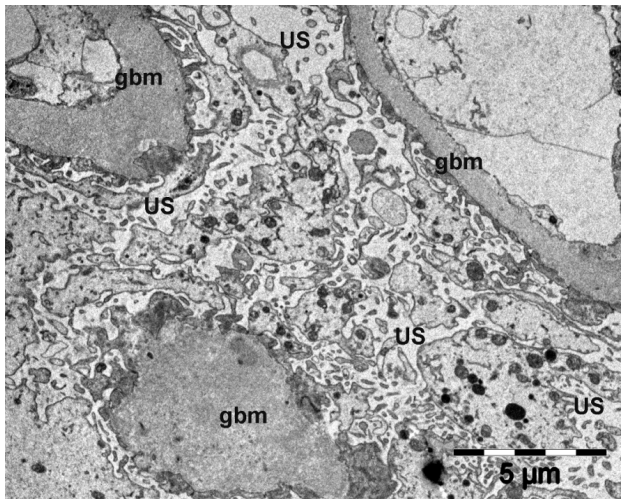

Figure 6 – ***Fragmented podocyte. The podocyte nucleus does not appear in the picture. The urinary space (US) is full with cytoplasmic fragments and microvilli. Glomerular basement membrane (gbm).***

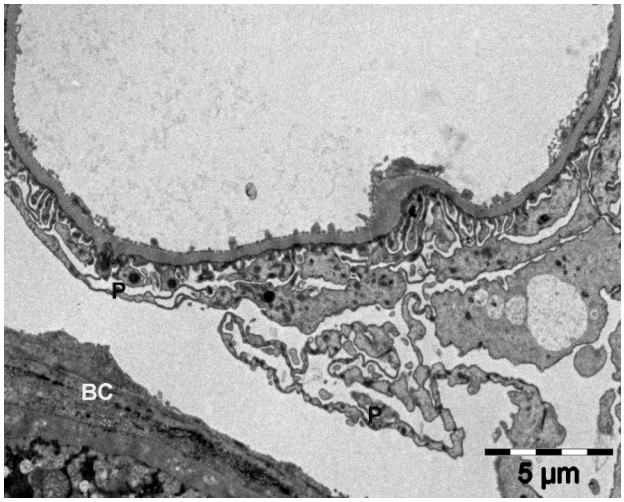

Figure 7 – ***Atrophic podocyte after 18 months of open hyperglycemia. Thin cytoplasmic processes (P) and normal foot processes. Bowman capsule (B).***

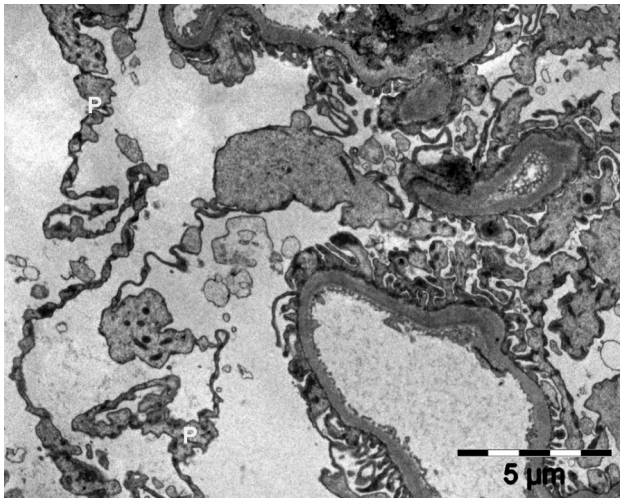

Figure 8 – ***Atrophic podocyte after 18 months of open hyperglycemia. The urinary space contains a few slender podocyte processes (P).***

When this atrophic transformation of podocyte processes reached a climax, foot processes were also affected by flattening and then effacement (Figure 9).

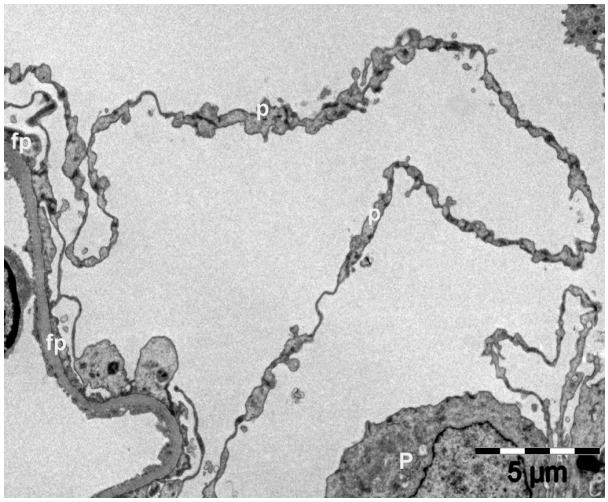

Figure 9 – ***Atrophic podocyte after 18 months of open hyperglycemia. Effaced foot processes (fp). Very slender primary podocyte processes (p). Podocyte body (P).***

Discussion

The podocytopathy is a complex of cellular lesions involving glomerular visceral epithelial cells, the podocytes. This complex is present in many glomerulopathies with specific characteristics for each kidney disease. The diabetic podocytopathy is believed to be the origin of microalbuminuria and proteinuria specific for the diabetic nephropathy.

In the present study, the earliest podocytes transformation was hypertrophy with a cytoplasm reach in organelles involved in the protein synthesis and occurred after the first 12 months. This structural remodeling denotes hyperactivity consecutive to the hyperglycemia through a series of cytokines. This hyperactivity sooner or later induces slit diaphragm injuries. From the ultrastructural point of view this event is marked by foot processes flattening and later effacement, associated with slit diaphragm reduction and increased permeability. Associated with the pedicel effacement, microvilli appeared in the urinary space. Microvilli occurrence is a current reaction to the foot process loss. Although these microvilli seem to be independent, small fragments on a thin section, they have to be connected to some cytoplasmic bodies; otherwise, they should be soon eliminated by the glomerular filtrate. Such features have been previously reported in human and animal studies of podocyte damage [10, 11].

This hypertrophic stage is later followed by a decrease of the podocytes volume and amount of organelles in the cytoplasm compartments. Thus, the primary processes became thinner and thinner and the foot processes were totally effaced.

Beside these atrophic features of podocytes, the fragmentation also occurred. All these aspects denote an atrophic evolution of the podocytopathy, meaning the decrease of cell number, and the slit diaphragm deterioration [12].

Apoptotic features of podocytes were not observed in this study, probably due to their rapid elimination by the increased amount of primary urine.

Conclusions

The diabetic podocytopathy is debuting with cell hypertrophy. The podocyte hyperactivity induces slit diaphragm injuries. This hypertrophy stage is later followed by the podocytes atrophy. The cytoplasm volume and organelles number are decreasing. The podocyte primary processes become very slender and the pedicels disappear. There is also a process of podocyte fragmentation and detachment. All these events contribute to the slit diaphragm damage and the increasing proteinuria.

Acknowledgments

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