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3. PODOCYTE INJURY IN GLOMERULAR DISEASES

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Podocytes are injured in diabetic and non-diabetic renal diseases (Figure 3.1. The spectrum of podocyte diseases).

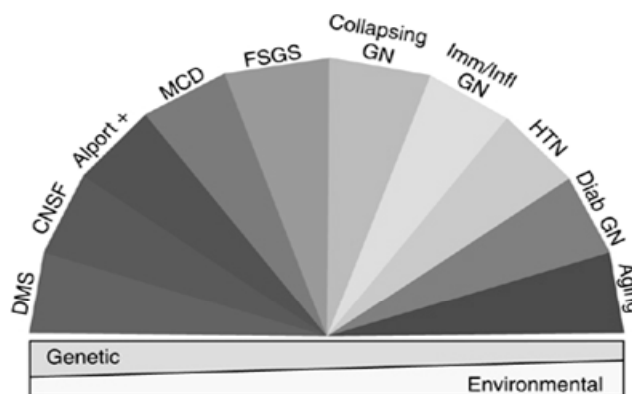


Figure 3.1. The spectrum of podocyte diseases. (6).

Together with glomerular endothelial cells (GEN) and glomerular basement membrane (GBM), podocytes form the glomerular filtration barrier in the kidney (Figure 3.2.). Podocyte damage or loss is an early symptom of many kidney diseases presenting clinically with proteinuria with or without nephrotic syndrome and renal failure owing to glomerulosclerosis. Injury to other components of the glomerular filtration barrier, such as GEN and GBM, may also present with nephrotic syndrome, proteinuria and renal failure, which suggests podocyte injury is not the only cause of those abnormalities.

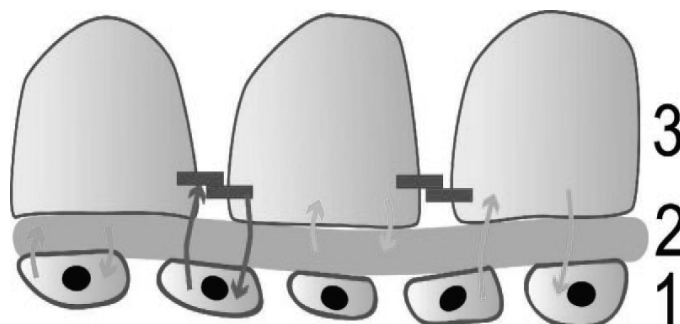


Figure 3.2. Glomerular filtration barrier. 1. Fenestrated endothelium; 2. glomerular basement membrane; 3 podocyte foot processes and slit diaphragm

The response of podocytes to injury is determined by their structure and function.

3.1 Podocyte structure

Podocytes are terminally differentiated (postmytotic) epithelial visceral cells with a unique and complex cellular organization. With respect to their cytoarchitecture, podocytes consist of three different segments: the voluminous cell body, major processes, and interdigitating foot processes. The foot processes are separated by filtration slits (slit pores), bridged by the thin slit membrane called slit diaphragm, a meshwork of proteins anchored at the sides of the foot processes. The cell body is located in the center of the cell bulging into the urinary space. Like an octopus, the podocyte cell body emits thick extensions. These structures - long primary processes branch to form the foot processes - pedicels that cover the surface of the glomerular capillary loops like the interdigitating fingers of two. The cytoskeleton composed of microfilaments comprises actin, myosin, α actinin, talin, paxillin and vinculin serving as podocyte backbone, not only maintains the shape of podocytes but also enables its continuous forming and adapting. This cytoskeleton supports the glomerular capillary wall and opposes the high hydrostatic pressure necessary for glomerular filtration. There are two actin cytoskeletal networks in foot processes: dense actin bundles above the level of the slit diaphragm running parallel to the longitudinal axis, and a cortical actin network just below the plasma membrane of the foot processes. The actin cytoskeleton is linked with other actin-binding proteins.

According to its molecular structure the podocyte has three domains: junctional, apical and basal (Figure 3.3.). The change in podocyte shape, called effacement, is not simply a passive process following injury, but occurs owing to a complex interplay of proteins that comprise the molecular anatomy of the different protein domains of podocytes.

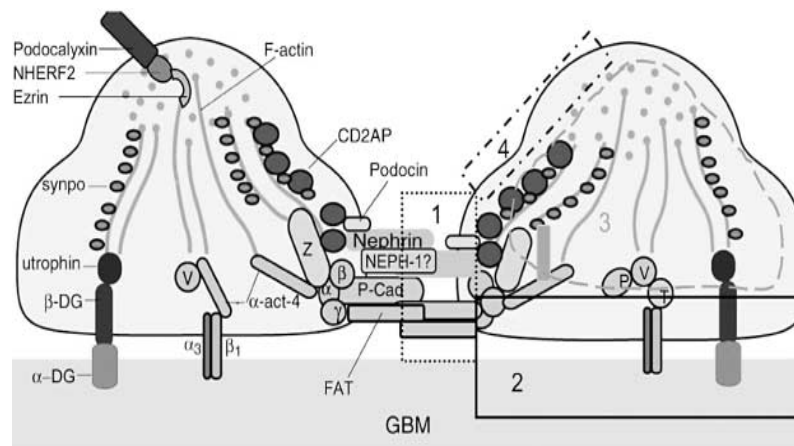


Figure 3.3. The three boxes (1-3) in the right half define the three domains of the foot processes: 1. junctional - slit diaphragm complex; 2. basal domain and GBM; 3. apical plasma domain with

glycocalyx. Interference with any of these three domains can cause foot process effacement and nephrotic syndrome.

The *junctional domain* encompasses slit diaphragm a complex of proteins located in the extracellular space, bridging adjacent foot processes. Tryggvason discovered the first slit diaphragm protein, nephrin. Nephrin is a transmembrane protein type I with both structural and signaling function. The cytoplasmic tail of nephrin binds to podocin. Nephrin also interacts with and localizes to CD2AP. The discovery of several proteins and their mutation analysis, including FAT, Neph-1, which interacts with nephrin, podocin, and CD2-associated protein, Neph-2 and -3, and densin, has emphasized the critical role of the slit diaphragm in maintaining normal function of the glomerular filtration barrier. By forming the only connection between adjacent podocytes, the slit diaphragm limits protein leakage by acting as a size barrier, analogous to a sieve. The slit may also function as a charge barrier, as some of these proteins are phosphorylated. Moreover, certain slit diaphragm proteins actively participate in podocyte signaling, thereby enabling the slit to communicate with other podocyte proteins such as the actin cytoskeleton (Figure 3.4.)

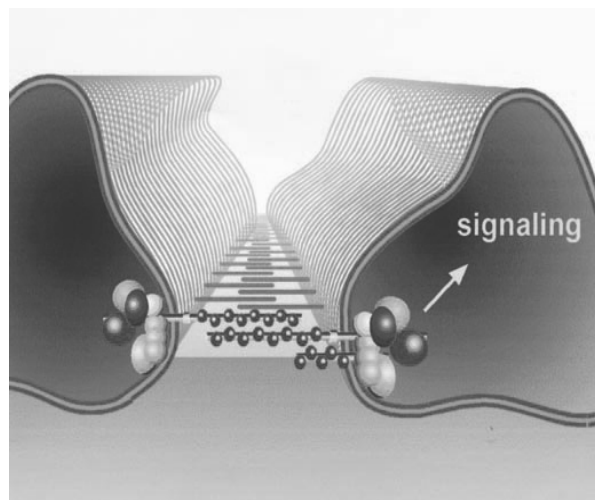


Figure 3.4. The slit diaphragm of podocytes is a specialized cell junction with signaling properties. It connects interdigitating foot processes serving as an essential part of the glomerular filtration barrier. Slit diaphragm proteins (nephrin and neph1) recruit cytoplasmatic adaptor proteins to initiate signal transduction.

The *apical membrane domain* located on the podocyte luminal side (facing the urinary space) has a negatively charged surface coat, owing to the presence of the surface anionic proteins podocalyxin, podoplanin, and podoendin. This serves two functions. First, negative charge limits the passage of negatively charged proteins into urinary space and second, it prevents parietal cell adherence to podocytes and keeps adjacent podocytes separated. Another important molecule on the luminal membrane is GLEPP-1, which has a possible receptor function. The *basal domain* is required to anchor podocyte to the underlying GBM. $\alpha 3\beta 1$ integrin and α and β - dystroglycans serve this function, and connect podocyte body to certain matrix proteins within GBM.

3.2 Podocyte functions

The podocyte has many functions: 1) it acts as a size and charge barrier to proteins; 2) supports the glomerular capillary wall maintaining the capillary loop shape; 3) opposes the high intraglomerular hydrostatic pressure; 4) provides synthesis and maintenance of the GBM; 5) produces and secretes VEGF required for GEN. The impairment of any of these functions following podocyte injury results in proteinuria and possibly renal failure.

3.3 Podocyte reaction to injury

The podocyte reaction to injury or damage in many diseases known as *foot processes effacement* is also called fusion, retraction, or simplification and *changes in podocyte number*. The foot processes effacement is characterized by flattening of foot processes due to gradual simplification of the interdigitating foot process. The whole podocyte looks flat due to retraction, widening, and shortening of the processes of each podocyte. (Figure 3.5.). The frequency of filtration slits is reduced, giving the appearance of a continuous cytoplasmic sheet covering the GBM. According to experimental data foot processes length decreases up to 70% and the width increases up to 60% compared to normal finding. This process is not a simple passive phenomenon but rather an energy dependent event. It is initiated by rearrangement of the *podocyte actin cytoskeleton*. Normally, actin cytoskeleton determines the shape of podocytes. Therefore, any disarrangement in actin or in actin-regulating proteins might lead to a change in podocyte shape and consequently function.

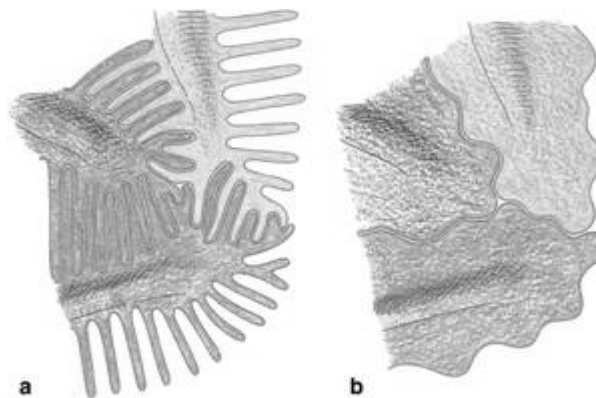


Figure 3.5. Foot processes effacement. a) normal; b) foot processes effacement

The cytoskeleton can be altered by at least four different mechanisms:

1. Systemic or locally produced toxins, viral infection, and local activation of the renin-angiotensin system
2. Abnormalities of cytoskeleton structural proteins α -actinin-4 and synaptopodin can adversely affect cytoskeletal dynamics. For example hereditary autosomal dominant FSGS is caused by mutation in α -actinin-4 increasing the affinity for actin and resulting in a change in cytoskeletal fluidity.

3. Congenital or acquired disorders can injure the slit diaphragm proteins deranging actin and nephrin signaling and leading to cytoskeletal reorganization. Mutation in slit diaphragm protein podocin causes autosomal- recessive steroid resistant nephrotic syndrome and FSGS in children. Benzing *et al.* showed that slit diaphragm proteins send signals regulating the podocyte polarity, survival, and cytoskeleton organization. Several studies have elucidated complicated signaling pathways by which specific slit diaphragm protein regulates the actin cytoskeleton interacting with proteins outside the slit diaphragm.
4. Experimental data (laminin β deficient mice) point to changes in the GBM structure as a cause of cytoskeletal derangements.

As already mentioned, actin cytoskeleton determines the shape of podocytes. Proteins regulating the actin cytoskeleton are of utmost importance for podocyte function, as any abnormality in actin-regulating proteins or actin itself might alter the shape and consequently the function of podocytes. Each of podocyte domains (junctional, apical and basal) as well as actin-associated proteins, synaptopodin and α -actinin-4 are linked to actin cytoskeleton enabling their interaction which, in a pathological setting, might lead to a rearrangement of the *podocyte actin cytoskeleton and foot processes effacement*.

Mutation and abnormalities in the slit diaphragm proteins in *junctional domain* (nephrin, podocin, CD2AP, and others) are associated with the rearrangement of the podocyte actin cytoskeleton and consequently foot processes effacement.

The *basal domain* is also important in maintaining the shape of podocyte. Two basal domain constituents, $\alpha 3\beta 1$ integrin and α and β dystroglycans, serve as matrix receptors for podocytes, anchor podocyte to GBM and mediate podocyte cell-matrix interaction. Experimental studies have shown that splitting of dystroglycans - matrix interaction and blocking $\beta 1$ integrins with an antibody, is associated with foot processes effacement.

Apical domain and foot processes effacement. The anionic apical proteins podocalyxin and podoplanin repel anionic proteins and prevent their passage into Bowman's space. Experimental data show that neutralization of the anionic surface charge leads to foot processes flattening and exposure to hyperglycemia suppresses the levels of podocalyxin. Podocalyxin overexpression inhibits cell-cell adhesion, and maintains an open filtration pathway between neighboring foot processes, i.e. neutralizing the anionic surface charge influences cell-cell adhesion and junctional permeability. There is experimental evidence linking podocalyxin directly to actin cytoskeleton, emphasizing that podocalyxin is needed to maintain foot processes shape. In summary, podocalyxin maintains podocyte shape, and a decrease in levels or loss of anionic surface charge leads to podocyte shape changes - foot processes effacement and distortion of the slit diaphragm, both leading to proteinuria. Similarly, studies have shown that injecting rats with an anti-podoplanin antibody results in podocyte effacement and proteinuria.

Changes in podocyte number occur in renal disease and with aging. A decrease in podocyte number resulting from *apoptosis or detachment* from the GBM is found in diabetic as well as non-diabetic glomerular diseases. This abnormality is associated with proteinuria and glomerulosclerosis. The decreased number of podocytes leaves

areas of bare GBM, which are foci for adhesions to parietal epithelial cells and crescent formation. Namely, nude areas of GBM are bulging into urinary space because the podocytes opposing the intraglomerular pressure are lost. A syncial attachment is formed upon the contact of the nude prominent part of GBM with parietal epithelial cells leading to focal segmental glomerulosclerosis. The mechanism of podocyte detachment is not well known. One explanation is that abnormalities in integrins or dystroglycans normally responsible for podocyte adhering to underlying GBM, might be the cause of podocyte detachment. As a consequence of detachment podocytes and podocyte-specific proteins can be found and measured in the urine of patients with proteinuria but not in healthy people. Measuring podocytes and their products in the urine might be a better disease marker than proteinuria.

Apoptosis is the second cause of podocyte depletion. There is evidence that slit diaphragm proteins govern podocyte survival. The research has been focused on CD2AP showing that absence or reduction of CD2AP and mutation in nephrin is associated with increased podocyte apoptosis. To survive, podocytes must be attached to GBM and once they detach the apoptosis increases significantly. This notion directed the research to $\alpha 3\beta 1$ integrin and α and β dystroglycans, showing reduced podocyte survival with their alterations. TGF- β induces podocytes apoptosis as well as angiotensin II. Angiotensin II blockade not only reduces systemic and intraglomerular pressures, but also podocyte apoptosis, thereby minimizing podocyte loss. Reduction of systemic and intraglomerular pressure and podocyte apoptosis reduce proteinuria and glomerulosclerosis. Hyperglycemia induces apoptosis providing a plausible explanation why the number of podocytes is reduced in diabetes.

Podocytes are terminally differentiated cells that normally cannot proliferate. They do not change their phenotype in response to injury, rendering the *inability to proliferate* the third cause of podocyte depletion. However, there are experimental data showing that podocyte can change their phenotype and proliferate in experimental crescentic glomerulonephritis and HIV nephropathy. Nevertheless, the increased and the reduced podocyte numbers are detrimental to glomerular function. The mechanisms underlying the inability of podocytes to proliferate are being investigated.

In summary, owing to their unique and complex cellular organization and many functions, podocytes are the most vulnerable constituent of the glomerular filtration barrier. Their injury and dysfunction lead to progressive glomerular filtration barrier failure presenting as nephrotic or non-nephrotic proteinuria, glomerulosclerosis, and eventually as renal failure. Podocyte injury leads to rearrangement of the *podocyte actin cytoskeleton* and *foot processes effacement*. The major causes (genetic or acquired) of foot processes effacement are impaired formation of the slit diaphragm complex, abnormality of the GBM or adhesion of podocytes to the GBM, abnormalities in the cytoskeleton or associated proteins, and alterations in the apical membrane domain of the podocyte.

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