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How Is Proteinuric Diabetic Nephropathy Caused by Disturbed Proteostasis and Autophagy in Podocytes?

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Progression of diabetic nephropathy (DN) is commonly defined by an increase in albuminuria from normoalbuminuria to microalbuminuria and from microalbuminuria to macroalbuminuria. Although many therapeutic interventions, including reducing hyperglycemia and intraglomerular pressure, have been shown to slow down the progression of DN, many patients still develop end-stage renal disease. A major difficulty in inducing remission in patients with early DN is the identification of biomarkers that could help to identify patients more likely to progress to end-stage renal disease. Traditional risk factors, such as albuminuria, do not effectively predict DN progression, and other predictors of DN have yet to be characterized and validated. The need for discovering sensitive and robust biomarkers to monitor the decline in renal function and to separate progressors from nonprogressors of DN is therefore of paramount importance.

Next to mesangial extracellular matrix deposition and a thickening of basement membranes, progressive loss of glomerular pericytes and "podocytes" and microvascular alterations appear to most closely correlate with the functional renal decline in DN (1–3).

Autophagy ("self-eating" in Greek) is a highly regulated lysosomal protein degradation pathway that removes protein aggregates and damaged or excess organelles in order to maintain intracellular homeostasis and cell integrity (4–6). This process was first described in 1957 by Sam Clark Jr. (7), but the term "autophagy" was coined in 1963 by Christian de Duve (8). Autophagy process is well conserved in the evolution from yeast to mammals in various cell types in many organs (9,10). The formation of autophagosomes depends on several genes including *Map1lc3B/LC3B*, *Becn1/Beclin-1*, and other autophagy-related (*Atg*) genes (4). Dysregulation of autophagy is involved in the pathogenesis of a variety of metabolic and age-related diseases (11–17). One caveat of many clinical studies dealing with tissues that should be taken into consideration is that there is a general tendency to extrapolate information regarding the levels of autophagy substrates to the levels of autophagy flux within the tissues. Despite the scarce but compelling literature on the roles of autophagy in the resistance to DN, studies need to determine whether autophagy genes and markers are suitable biomarkers or targets for therapeutic intervention to ameliorate the progression of DN. Future research would ultimately determine the most reliable method for alterations of autophagy related to DN progression, taking ease and quantity of sample acquisition into account.

The function of autophagy in the kidneys is currently under investigation, and it has been shown to have a renoprotective effect in several animal models of aging and acute kidney injury, especially in glomeruli (18–21). Importantly, postmitotic podocytes exhibit high levels of basal autophagy as a key regulator of podocyte and glomerular maintenance (22) (Fig. 1).

In this issue of *Diabetes*, Tagawa et al. (23) confirm the protecting role of podocyte autophagy on DN, as selective targeting of the *Atg5* alleles in these cells accentuated experimental high-fat diet–induced DN as previously observed in a model of type 1 DN (24). Furthermore, it is interesting that the loss of autophagy in podocytes affects the ultrastructure and function of these cells but also that

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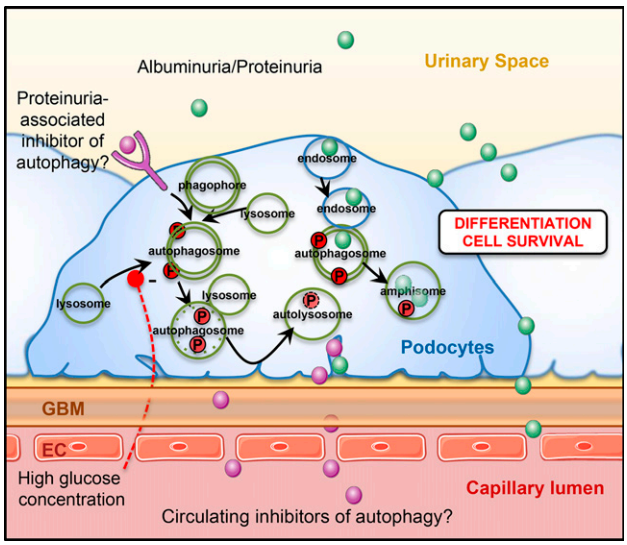

Figure 1—Postmitotic podocytes exhibit high levels of basal autophagy as a key regulator of podocyte and glomerular maintenance. Elevation of glucose concentration inhibits podocyte autophagy (—). Selective targeting of the *Atg5* alleles in these cells disrupted autophagosome formation and accentuated experimental high-fat diet–induced DN. Altered autophagy led to accumulation of damaged organelles and proteins and disturbance of lysosomes trafficking. Furthermore, insufficient podocyte autophagy was found in diabetic patients and rats with massive proteinuria accompanied by podocyte loss, but not in those with no or minimal proteinuria. The nature of the negative signal associated with albuminuria is not known. Endocytosed albumin (green dots) per se may saturate podocyte autophagy or albuminuria may be associated with local passage or secretion of inhibitory mediators (purple dots) that remain to be identified. Such circulating mediator may also impact endothelial autophagy that has also been shown to limit progression of experimental DN. Red dots represent phosphatidyl-ethanolamine–conjugated microtubule-associated protein 1A/1B-light chain 3 (LC3), which is recruited to autophagosomal membranes and helps to monitor autophagy flux. EC, endothelial cells; GBM, glomerular basement membrane.

of nearby mesangial cells, which become sclerotic. These data underline the communication between podocytes and mesangial cells, which deserve further studies in the field of glomerular diseases.

Importantly, Tagawa et al. (23) also show insufficient podocyte autophagy in patients with diabetes and rats with massive proteinuria accompanied by podocyte loss but not in those with no or minimal proteinuria. The causes of such alteration of podocyte autophagy are potentially several. Meanwhile, stimulation of cultured podocytes with sera from patients with diabetes or rats with massive proteinuria-impaired autophagy resulted in apoptosis. These findings might be of crucial importance as they suggest the existence of serum factors promoting podocyte stress and dysfunction with blunting of autophagy in proteinuric individuals. Let us hope this provocative finding will lead to future work aimed at identifying such factors. Another question rising from the study by Tagawa et al. is whether inhibition of autophagy by serum from proteinuric diabetic animals or patients with diabetes would also occur in other critical cell types such as endothelial cells. In fact, endothelial autophagy was recently shown to be of critical importance for the limitation of progression of DN (24). There is a strong relationship between endothelial dysfunction and DN in humans (25,26), and an increase in urinary albumin excretion in combination with rising blood pressure is a major risk factor for cardiovascular morbidity (27–30). The mechanism for such cross talk between the glomerular filtration barrier and other vascular beds is unclear and represents a major pathophysiological question.

Fundamentally, the work by Tagawa et al. (23) also adds a major question mark about the pathophysiological importance of protein homeostasis, also called "proteostasis," in terminally differentiated cells such as podocytes. Different mechanisms are involved in proteostasis, among them degradation systems (the main intracellular proteolytic systems being proteasome and lysosomes), folding systems (including molecular chaperones), and enzymatic mechanisms of protein repair (31). Tagawa et al. report the accumulation of large lysosomes with an increase in lamp2-positive areas and an absence of autophagosomes in podocytes from massively proteinuric and diabetic rats and in mice with genetic targeting of *Atg5*, a gene required for autophagy. Thus, metabolically and genetically driven autophagy deficiency was associated with dysfunctional lysosomes accumulated in podocytes. The preservation of lysosome- and autophagy-mediated proteostasis may be critical for podocytes to cope with increased amounts of damaged proteins produced during diabetes, a condition favoring increased intensity of nonenzymatic modifications of amino acids (32) and high incidence of posttranslational modifications of proteins as reported in β-cells and nerves (33,34).

In conclusion, the study by Tagawa et al. (23) adds to the recent corpus of evidence that therapeutic stimulation or at least maintenance of autophagy and proteostasis represents an important nascent field of research to prevent and treat complications of diabetes.

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