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Protecting podocytes: how good do we need to be?

Kevin V. Lemley[1]

Progression of many glomerular diseases has been firmly tied to a loss of podocytes, followed by a deterioration of glomerular architectural stability eventuating in segmental, and ultimately global, sclerosis. Recent studies have begun to clarify the nature of the autonomous (disease-independent) aspects of this process, as well as to explore mechanistically the 'unreasonable effectiveness' of angiotensin blockade in slowing glomerular disease progression. Quantitative monitoring of podocyte loss (e.g., to assess therapy) remains a challenge.

Kidney International (2012) **81,** 9–11. doi:10.1038/ki.2011.329

The first indications of the importance of podocyte loss in the progression of kidney diseases came from animal models[1] and from cross-sectional studies of human disease.[2] The concept of podocyte 'insufficiency,' first clearly enunciated by Rennke and co-workers,[1] was further developed by Kriz and co-workers[3,4] into the current canonical model for the development of glomerular sclerosis: a loss of podocytes leads to 'bare areas' of glomerular basement membrane, which in turn lead to formation of synechiae to Bowman's capsule and then to segmental and finally global glomerular sclerosis.

More recently, ingenious animal models have been developed in the mouse[5] and the rat[6] that allow a much more precise titration of the degree of podocyte loss than was possible with previous models using podocyte toxins such as puromycin or adriamycin. This has allowed those aspects of glomerular disease progression due solely to reduced podocyte number to be studied in isolation. In some sense, this represents the intraglomerular analog of the subtotal nephrectomy model introduced decades ago to study the effects of reduced nephron number on renal disease progression. Also analogous to the subtotal nephrectomy model is the existence of an apparent threshold level of initial podocyte loss necessary for the subsequent development of an autonomous phase of podocyte loss.

Fukuda and colleagues[7] (this issue) now present a meticulous study of the natural history of the autonomous progression of podocyte loss and consequent glomerular sclerosis, and their modulation through angiotensin blockade. The study is based largely on the authors' technique of targeting podocytes for killing by diphtheria toxin (DT) in a transgenic rat model in which the human DT receptor (hDTR) is expressed under the control of the (human) podocin promoter.[6] The rodent analog of the hDTR does not bind DT, so wild-type rats are naturally resistant to its cytotoxic effects. Since, under the control of the podocin promoter, the hDTR is expressed only in podocytes of the transgenic rats, it is only these cells that take up and are killed by administered DT. Different levels of initial podocyte loss can be achieved by varying doses of DT. Other rat models (subtotal nephrectomy, puromycin) support the generality of Fukuda and colleagues'[7] findings in this model.

The authors estimate glomerular podocyte number by two complementary methods: the thick-and-thin-section method to determine the number of WT-1-positive nuclei (podocytes) per unit glomerular volume, and the fraction of glomerular tuft staining for GLEPP1, a podocyte-expressed protein. In addition, the authors use a surrogate indicator for urinary podocyte loss—mRNA for the podocyte proteins podocin and nephrin in the urinary sediment. These more novel indicators of progression are correlated with the incidence of glomerular sclerosis and quantitative proteinuria.

Fukuda and colleagues[7] show that a process of autonomous progression—based on ongoing podocyte loss—follows the initial loss of a threshold fraction of podocytes (greater than about 30%) induced by DT. Ongoing loss of podocytes 'destabilizes' the glomerulus, leading to glomerular sclerosis. Progression can be prevented by combined angiotensin blockade (with enalapril and losartan). Interestingly, the effect of angiotensin blockade on proteinuria is quite rapid (presumably reflecting improved podocyte function), whereas beneficial effects on urinary podocyte excretion (as reflected in urine podocin mRNA) are delayed by 2 weeks. If angiotensin blockade is stopped, the several indices of autonomous progression recommence.

It has long been known that angiotensin blockade is 'unreasonably effective' in preventing progression of many renal diseases. So what does this study add to our understanding of how angiotensin blockade prevents progression? The most significant finding is that prevention of autonomous

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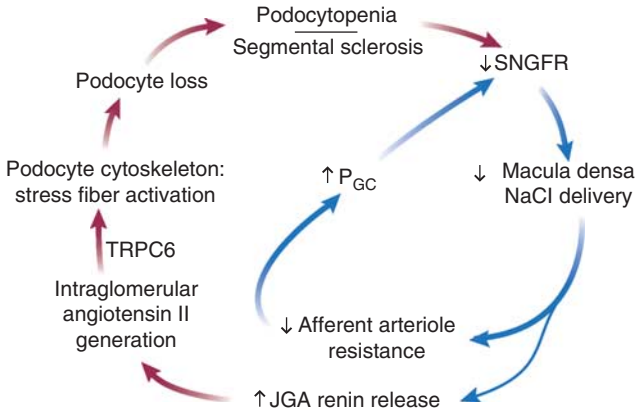

Figure 1 | Mechanism for autonomous ongoing loss of podocytes once a threshold number are lost. Loss of podocytes and segmental sclerosis leads to decreased ultrafiltration capacity and single-nephron glomerular filtration rate (SNGFR). Decreased macula densa NaCl delivery triggers decreased afferent arteriolar resistance and increased glomerular capillary pressure (P_{GC}); it also increases release of renin from the juxtaglomerular apparatus (JGA). Local production of angiotensin II within the glomerulus activates podocyte stress fibers (allowing podocytes to better counter the increased distending pressures in the glomerular capillaries) but also may lead to additional loss of podocytes in the long term. Blue arrows, homeostatic pathways; red arrows, injury pathways.

progression seems to be closely tied to amelioration of ongoing, secondary podocyte loss (as indicated by both urinary mRNA excretion and the structural indices of podocyte loss). This was the case independent of the treatment group, suggesting that 'off-target' effects of angiotensin blockade (for example, its antifibrotic effects) are of secondary importance in the broad phenomenon of renal disease progression.

Most importantly, how does the initial loss of a supra-threshold number of podocytes lead to a secondary, ongoing loss of podocytes? Here, the authors provide only evidence that this secondary mechanism is somehow dependent on the actions of angiotensin II.

A recent refinement[8] of the murine model[5] based on the podocyte-specific expression of the receptor (hCD25) for the immunotoxin LMB2 allows a somewhat cleaner approach to exploring the role of podocyte–podocyte interactions in the phenomenon of autonomous progression. In the latter version of the model, the transgenic mice are chimeric for hCD25-positive podocytes. Thus, the degree of podocyte killing is a function not only of the dose of toxin, but of the random expression of the hCD25 receptor in only some of the glomerular podocytes, potentially avoiding the complication of sublethal injury in podocytes that were not initially killed by toxin exposure. The non-susceptible podocytes are additionally labeled with a stainable marker, so they may be distinguished from hCD25-expressing podocytes (or from any 'replacement' podocytes that may have arisen from local niche populations).

A number of local (intraglomerular) mechanisms could explain the progressive, autonomous loss of podocytes resulting from an initial supra-threshold loss. Some of these have been considered by Ichikawa and colleagues under the rubric of 'podocyte damage damages podocytes.'[9] Such mechanisms envisage a podocyte-to-podocyte spread of injury, possibly due to the loss of antiapoptotic cell–cell signaling between adjacent, interdigitating podocytes via the slit diaphragm, autocrine danger or death signals coming from injured podocytes, or deleterious effects of local protein leakage.

An alternative, and non-exclusive, intraglomerular mechanism not related to podocyte–podocyte interactions, however, may better explain the therapeutic effectiveness of angiotensin blockade based on macula densa signaling and the tubuloglomerular feedback mechanism (Figure 1). As podocyte number decreases, either segmental parts of the glomerular tuft are lost to sclerosis, or a decreased number of podocytes must 'stretch' to cover the filtration surface, leading to broadening of foot processes. Either factor will lead to a decrease in the single-nephron ultrafiltration coefficient, lowering the single-nephron glomerular filtration rate (SNGFR). An attendant decreased delivery of NaCl to the macula densa will lead both to a decrease in afferent arteriolar resistance via tubuloglomerular feedback (in order to increase intraglomerular capillary pressures and support SNGFR) and to an increase in local renin release from the juxtaglomerular apparatus. Release of renin from the afferent arteriole results in local activation of the renin–angiotensin system within the glomerular tuft.[10] Increases in angiotensin II concentration within the glomerulus may affect the podocyte actin cytoskeleton (perhaps via activation of TRPC6 channels[11]), increasing podocyte stress fibers and effectively counterbalancing the increased intracapillary pressures, thereby preserving glomerular capillary structure. Although transient local activation of the renin–angiotensin system in this way may allow adaptive alterations in the podocyte cytoskeleton in the face of short-term increases in filtration pressures, long-term local angiotensin effects probably contribute to an ongoing loss of podocytes via a number of possible mechanisms. This may explain the 2-week lag period before angiotensin blockade seems to protect against podocyte loss into the urine.

One of the more exciting findings of Fukuda and colleagues[7] is the possibility that monitoring urinary mRNA excretion for podocyte-associated transcripts may give a reliable noninvasive view of the pivotal intraglomerular event in progression—ongoing podocyte loss. A 'liquid biopsy' has been a dream of clinical nephrologists for quite a while. More work is needed, however, before it can become a reality. Hara and colleagues[12] have shown that in addition to intact podocytes, the urine sediment contains fragments of apical cell membranes, possibly originating from shedding of vesicles from podocyte microvilli. It is not clear whether or not these fragments contain mRNA from their podocytes of origin. So urinary mRNA levels may reflect the loss of intact podocytes (live and apoptotic), changes in the mRNA content of these intact podocytes, and mRNA contained in podocyte fragments. The disparity between the urinary excretion patterns of nephrin and podocin raises the possibility that the determination of additional urinary mRNA species

of podocyte-expressed proteins might yield a richer and more informative view of podocyte injury and loss.

The study of Fukuda *et al.*[7] also raises the question of whether it is possible that, if initial therapy in human disease can limit early podocyte losses to less than a certain threshold,[13] inexorable progression to renal failure may be avoided. Conversely, if this is not possible, will it always be the case that aggressive enough angiotensin blockade (possibly, targeted to minimize urinary podocyte excretion) will be sufficient to preserve glomerular architectural stability, protect remnant podocytes, and assure long-term renal survival?

DISCLOSURE
The author declared no competing interests.

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Interpretation of genetic variants of uncertain significance in atypical hemolytic uremic syndrome

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Mutations in complement proteins predispose to atypical hemolytic uremic syndrome (aHUS). Mutation screening in aHUS is challenging, because most of the disease-associated mutations are individually rare, and a significant proportion of variants consist of missense mutations of unknown significance. The definitive interpretation of a variant of unknown significance (VUS) is often dependent on a reliable functional assay too time-consuming to be used in a diagnostic screening service. Allied research groups have analyzed these VUSs in aHUS.

Kidney International (2012) **81,** 11–13. doi:10.1038/ki.2011.330

Atypical hemolytic uremic syndrome (aHUS) is the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Advances in the past 15 years have shown aHUS to be a disorder of the alternative pathway of complement. Mutations in the complement-regulatory proteins factor H (*CFH*), factor I (*CFI*), and membrane cofactor protein (*CD46*; MCP) and complement components factor B (*CFB*) and *C3* all result in complement overactivation.[1]

Genetic screening of people with aHUS is now in mainstream clinical practice and provides important prognostic information that guides clinical decision making. With increasing genetic analysis, genotype–phenotype correlations have emerged. For instance, in patients with mutations in *CFH* there is a very poor prognosis; 60–70% of patients die or reach end-stage renal failure within a year. In comparison, those with *CD46* mutations have a better outcome, with about 80% remaining dialysis-independent.[2] The genetic defect can also predict the aHUS recurrence rate after renal transplantation.[3] The complement-regulatory defect in those with mutations in the membrane-bound *CD46* is corrected by an allograft bearing wild-type *CD46*. In those with mutations in the serum complement regulators *CFI* and *CFH*, which are produced by the liver, a renal transplant does not correct the defect, so the recurrence rate is high. Thus genetic screening determines the suitability for isolated renal transplantation, combined liver–kidney transplantation, or the prophylactic use of eculizumab for transplantation.

Mutation screening in aHUS is challenging because most of the disease-associated mutations are individually rare (Figure 1). In most cases, interpretation of the functional significance of nonsense mutations, large gene rearrangements, and frameshift mutations is clear-cut. However, a significant proportion of variants consist of missense mutations or potential splice-site changes of uncertain biologic or clinical relevance. These variants of unknown significance (VUSs) pose a challenge in reporting the genetic results. In these

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