Glomerular Homeostasis Requires a Match between Podocyte Mass and Metabolic Load

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In this issue of *JASN*, Fukuda and colleagues[1] report findings in rats with impaired mammalian target of rapamycin (mTOR) signaling, due to a transgene that acts as a dominant negative to impair mTOR signaling. The authors show that, in normal F344 rats, with increasing weight gain, podocyte volume increases but fails to keep pace with the even greater increase in glomerular volume. In transgenic rats, this imbalance becomes more marked when the ability of podocytes to undergo compensatory hypertrophy is blocked by the AA-4E-BP1 transgene, resulting in failure of the podocytes to adequately cover the glomerular basement membrane (GBM). The principle findings of this study include the following: AA-4E-BP1 transgenic rats progress to ESRD by 12 months, in a fashion directly correlated with transgene expression and body weight (note that laboratory rats gain weight throughout their lifetime, when give free access to food); the earliest morphologic lesions are denuded sections of GBM, lacking coverage by podocyte foot processes, and adhesions to the parietal epithelial cells, an early finding of focal segmental glomerulosclerosis (FSGS); the weight at which proteinuria, termed the weight-proteinuria threshold, is decreased by 40%–50% by uninephrectomy; and caloric restriction that stabilizes weight also prevents proteinuria and FSGS.

These intriguing findings lead the authors to propose a novel mismatch hypothesis that relates an imbalance between podocyte growth potential and somatic growth, resulting in the inability of podocyte coverage of the greater GBM surface area of an enlarged glomerulus, which in turns leads to proteinuria and glomerulosclerosis. This mismatch hypothesis could also be stated as a mismatch between podocyte mass, either reduced numbers or limited hypertrophic potential, and glomerular size, determined by interactions between body size, glomerular number, and other factors, initiating glomerular disease or accelerating other glomerulopathies.

The mTOR complex (mTORC1) fulfills a critical function for cellular homeostasis: to integrate various environmental signals (nutrients, cytokines, hormones, growth factors) and to determine macromolecular synthesis, a key factor in determining cell size, and supports DNA replication required for an increase in cell number. mTORC1 influences protein synthesis through two pathways: stimulation of S6 kinase and 4E-BP1. Fukuda and colleagues use a well-characterized, dominant negative construct (AA-4E-BP1) and expressed this transgene in a podocyte-restricted fashion. Active 4E-BP-1 binds the eukaryotic initiation factor (EIF)-4E, preventing the initiation of translation and promoting apoptosis. When mTORC phosphorylates and inactivates 4E-BP1, the released EIF-4E is able to bind capped mRNAs and promote their translation; all eukaryotic nuclear RNAs contain this methylated cap motif at the 5′ terminus.

The current study used a mutated 4E-BP1 in which alanine replaces serine 37 and threonine 46 (AA-4E-BP1); the mutant is constituitively active and therefore EIF-4E remains inactive. Upstream lies the TSC1/TSC2 complex, which by Ras homology enriched in brain (Rheb) determines the activation status of mTORC1. The tuberous sclerosis complex 1 (TSC1) and TSC2 complex are a critical node, activated by AMP-activated protein kinase (AMPK) and inactivated by AKT and extracellular signal-regulated kinases (ERKs). Further upstream of AMPK are a variety of cell surface receptor kinases that transduce signals from cytokines, growth factors, and hormones.

A current paradigm of glomerular pathophysiology, supported by two decades of clinical and laboratory research, states that podocyte depletion leads to progressive glomerulosclerosis, either as a primary process or as an accelerant of another glomerulopathy. Following recognition of the role of reduced glomerular number by Brenner *et al.*[2] as a risk for hypertension and progressive kidney disease, the central role of podocytes was proposed by Kriz *et al.*[3] Elegant podocyte depletion models developed by Ichikawa *et al.*[4] using transgenic mice and by Wiggins et al.[5,6] using transgenic rats demonstrated that podocyte depletion below a critical threshold results in glomerulosclerosis, and injury to certain podocytes propagates to other podocytes, likely because of the adaptive processes of cellular hypertrophy and ultimately glomerular hypertrophy placing stress on the initially unaffected podocytes.

Fukuda and colleagues hypothesize that in patients, the appearance of adaptive FSGS (to be distinguished from

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primary FSGS) depends on four factors: body weight and closely associated glomerular tuft volume, nephron number, podocyte hypertrophic response, and podocyte loss caused by disease. Hypothetically, all of these factors have many genetic and/or environmental determinants.

Metabolic load, as the term is used in this study, relates body size to glomerular stress, which in turn is defined as the propensity for glomerular enlargement, progressive podocytes loss, and glomerulosclerosis. What constitutes metabolic load and what is the afferent signal to the glomerulus? This remains unclear. A number of circulating cytokines could contribute. Obesity is associated with hyperinsulinemia, increased angiotensin II, aldosterone, plasminogen-activator inhibition (PAI-1), and altered adipokine levels, including decreased adiponectin.[7] These and other growth factors bind receptors that in some cases lie upstream of mTOR, which as shown by Fukuda and colleagues, lies in the signaling pathway that drives podocyte hypertrophy. Hyperlipidemia may also contribute, as may other metabolic alterations. Further exploration into the metabolomics of obesity and the relationship of various metabolites to glomerular growth may yield additional insights. Finally, systemic hypertension is likely one part of how a big body stresses the kidney; in the present study, although BPs were noted to be similar in 100-g rats of wild-type and transgenic genotype, BP was not measured as the rats gained weight and likely became hypertensive.

In humans, obesity is associated with progressive glomerular disease, including obesity-related glomerulopathy with proteinuria and obesity-related adaptive FSGS. Although these diseases affect a small subset of the obese population, they are becoming more prevalent as the population of obese individuals increases, particularly those with more severe obesity.[8,9] Thomas *et al.*[10] performed a meta-analysis of 11 studies involving 30,146 subjects and found that metabolic syndrome was associated with an odds ratio of 1.55 (95% confidence interval: 1.34, 1.80) for CKD (estimated GFR <60 ml/min per 1.73 m^2), with increasing risk associated with an increasing number of metabolic syndrome criteria present. Furthermore, increased body size caused by extreme muscle development[11] and growth hormone excess[12] is also associated with glomerulomegaly and FSGS.

Are there clinical implications of these new basic science findings? The relationship between obesity and CKD is complex, because obesity is associated with increased body size, metabolic syndrome, and chronic inflammation, and each component could plausibly contribute to glomerular stress. The findings of Fukuda *et al.* provide a mechanistic rationale in support of the existing recommendations to individuals at risk for CKD to avoid becoming obese and for individuals with established CKD who are obese to lose weight, as reviewed recently.[13,14]

In conclusion, these new data add a new dimension to our understanding of podocyte response to stress. Increased body size places an increased demand on glomeruli, and the renal response depends on *podocyte number* (there is limited capacity to replenish or increase podocyte cell numbers by stem cells within the parietal epithelium) and on *podocyte hypertrophy* (which is impaired in the model presented here); whether genetic variation in humans confers a similar phenotype remains to be determined. Thus, increases in metabolic load promote glomerulomegaly, which requires that increases in podocyte mass (podocyte number $\times$ average size) to maintain normal glomerular cytoarchitecture. When there is reduced podocyte number (loss caused by disease or impaired replacement) or reduced capacity for podocyte hypertrophy, the failure to adequately cover the glomerular basement membrane initiates a chain of events leading to FSGS.

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DISCLOSURES

None.

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See related article, "Growth-Dependent Podocyte Failure Causes Glomerulosclerosis," on pages 1351–1363.

Dying Cells and Extracellular Histones in AKI: Beyond a NET Effect?

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The sequelae of tissue injury caused by hypoxia, ischemia, mechanical stress, or pathogen-induced inflammation are initiated in part by the release of danger/damage-associated molecular patterns (DAMPs) from damaged or dying cells.[1] Typically serving normal functions in cell homeostasis within the cell, these endogenous molecules are recognized as danger signals by cell surface receptors when released into extracellular spaces. In AKI, as well as other kidney disorders and injury to other organs, the ensuing inflammation and recruitment of the immune system contribute to the pathogenesis and resolution of disease. A wide variety of candidate DAMPs and their putative receptors have been identified, and their role in kidney disease is gradually being uncovered[1]. While some receptors appear to be selective for DAMPs, others such as toll-like receptors (TLRs)[2,3] respond to DAMPs and are included among pattern recognition receptors for pathogen-associated molecular patterns (PAMPs). DNA extruded from apoptotic or necrotic cells falls in one of the DAMPs categories, but it also is becoming clear that associated nuclear molecules, such as histones, contribute to tissue injury and disease. In the current issue of *JASN* Allam *et al.*[4] investigate whether histone release from dying renal cells functions like DAMPs and contributes to AKI in a TLR-dependent manner.

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The appearance of histones in the extracellular space is not well understood but may arise from apoptotic or necrotic cells through passive release (perhaps as dying cells release their cell contents), from proinflammatory cells by active secretion, or as a component of neutrophil extracellular traps (NETs) from infiltrating neutrophils. In a cell death process termed NETosis that seems largely to be involved with clearance of invading pathogens, nuclear DNA and associated proteins (such as histones), some of which are antimicrobial, are extruded from neutrophils into the extracellular space to form fibrous networks, called NETs.[5] Neutrophils are often the first responders to pathogens and tissue injury and are recruited to inflammatory sites to contain infections by a variety of means, including NETosis.[6] NETs serve important antibacterial functions, but histones (and perhaps other NET components) may also produce collateral damage to host bystander cells. Although the term NET was originally named for and described in neutrophils, extracellular traps containing DNA and proteins are also released from other cells, including monocytes/macrophages and eosinophils[7] and may serve as a more generalized defense mechanism. Indeed, extracellular traps and histones, in particular, may play a role in a variety of inflammatory and autoimmune disorders, such as stroke,[8] systemic lupus erythematosus,[9] thrombosis,[10] and AKI, although the latter has not yet been examined.

To explore the role of histones in kidney injury, Allam *et al.* conducted a very thorough series of experiments to demonstrate that histones, when released by damaged cells, mediate cell death and inflammation and contribute importantly to postischemic and septic acute kidney.[4] They first demonstrated that dying tubular epithelial cells release histones and that histones act directly on renal epithelial and endothelial cells grown in culture to induce both apoptotic and necrotic cell death. The pathophysiological relevance of extracellular histones was explored in mice. Direct visualization of local microcirculatory events was established through *in vivo* microscopy of the mouse cremaster muscle in which local application of histones enhanced leukocyte migration and adherence, and immunostaining showed transendothelial migration of neutrophils and monocyte/macrophages. Chemokine-induced chemotaxis and adhesion molecule-induced rolling, adhesion, and transmigration of leukocytes mediate these processes.[11,12]

Next, Allam *et al.* injected histones directly into the kidney by intra-arterial injection, which led to inflammation, necrosis, and increased proinflammatory cytokine expression in the injected kidney that was prevented by digestion of the histone preparation with activated protein C, a commonly used method for confirming histone-specific effects. The deleterious effects of histones depended on pretreatment of mice with a low dose of LPS to induce TLR2 and TLR4 expression and were absent in TLR2/4 double-null mice, suggesting a role in septic AKI. In an effort to demonstrate cause and effect, the authors injected blocking antibodies and showed that neutralization of extracellular histones, and specifically of