Diseased renal glomeruli are getting soft. Focus on “Biophysical properties of normal and diseased renal glomeruli”

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Tissues and cells are characterized by mechanical properties that are variously described as elasticity, deformability, or stiffness, which are critical components in determining their normal structure and function (reviewed in Ref. 25). However, biologists and physicans are not used to thinking of mechanical signals as having a primary role equivalent to biochemical signals in normal biology and disease. Appreciation of the importance of mechanical factors in biology, including the elastic properties of tissues and cells, has increased over the past 20 years. In many situations, mechanical factors, in collaboration with biochemical signals, have primary roles in the development and maintenance of tissue phenotype as well as disease. In this context, change from a soft (deformable ~0.2 kPa) to a hard/dense (nondeformable >4 kPa) breast tissue represents a major risk factor for developing breast cancer, and females with higher levels of breast density are at higher risk for developing breast cancer (1). This is probably due to the fact that normal breast cells exposed to a stiff/hard extracellular environment (i.e., collagen-rich environment) lose their epithelial morphology as well as the ability to form acini and tend acquire a more invasive/malignant phenotype (18). Another example of how the elasticity of the surrounding environment determines cell behavior is offered by the liver. The elastic modulus of normal liver is ~0.5 kPa but can increase to ~15 kPa in the course of injury or fibrosis (6, 23). As a consequence of increased matrix stiffness, hepatocytes, stellate cells, and portal fibroblasts divide more rapidly, thus contributing to the disease (15). A third example is from studies showing that neurons grow selectively when brain cortical cells are plated on soft substrates (0.15–0.3 kPa) (7, 8). However, when the same cells are plated on more rigid substrate (2 kPa), glia grow selectively (7, 8). Thus it is clear that the mechanical properties of the extracellular environment are important contributors to cell lineage, cell proliferation, cell migration/invasion, and ultimately disease phenotypes. In addition to extracellular elasticity, intracellular elastic properties play a key role in determining cell fate and function. Intracellular elastic properties are determined by factors that include: 1) the amount and form of polymerized actin, 2) the extent and nature of polymerized actin cross-linking by other proteins (α-actinin), 3) adhesion to substrate (focal adhesions, type of matrix receptors), and 4) the ability of a cell to develop internal tension (changes in the organization of the cytoskeleton, adhesion complexes, microfilaments, and myosin). Changes in any of these factors might contribute to alterations in intracellular elastic properties, cell behavior, fate, and ultimately tissue damage (reviewed in Ref. 5).

Little is known about the biomechanical properties of healthy and diseased kidneys. Although pathologists know that kidneys from patients with end-stage kidney disease are hard and dense, and nephrologists who perform biopsies know that often a diseased kidney repels a biopsy needle, it is unknown whether changes in stiffness or elasticity directly contribute to the pathogenesis of kidney disease. The glomerulus, the filtering unit of the kidney, is a frequent site of injury and progressive disease. Glomeruli consist of 1) specialized fenestrated endothelial cells; 2) mesangial cells; 3) terminally differentiated visceral cells (podocytes); and 4) the glomerular basement membrane (GBM) that separates podocytes from endothelial cells (see also Fig. 1A). In humans, the kidneys filter ~1.5 liter of blood per minute, which means the glomeruli are exposed to pulsatile flow and must have the ability to adjust and/or quickly respond to changes in blood volume and/or pressure to maintain a constant filtration rate. With loss of renal mass, glomerular capillary pressures increase, and this increase in pressure appears to be related directly to injury of presumably structurally normal capillaries.

A number of human genetic glomerular diseases and mouse models of genetic disease have been described that could potentially be caused by changes in the mechanical/elastic properties of either the extracellular environment or glomerular cells. Many of the genes mutated in these renal diseases (e.g., the α3 or α5 chain of collagen IV, α-actinin-4, or podocyte-specific proteins that alter cell-cell junctions) affect the structure of either the GBM or the cytoskeleton of glomerular cells (9, 11, 13, 19, 20) (Fig. 1B). No evidence exists that the early stages of these diseases are characterized by abnormal (increased) glomerular capillary blood pressure or flow. Therefore, glomerular injury in these as well as other conditions [e.g., HIV-associated nephropathy characterized by altered podocyte structure (21)], involves abnormal, presumably weakened, structures in the presence of normal hemodynamic forces, and strongly suggests that in these conditions, cellular and tissue mechanical abnormalities may have a fundamental role in glomerular injury.

If altered mechanical characteristics of glomeruli contribute to disease, glomeruli should have specific mechanical characteristics with elastic moduli that need to be kept constant to allow proper function of these filtering units and maintain the differentiated state of glomerular cells. Whether these biophysical properties change in the course of glomerular disease and whether the elastic moduli of diseased glomeruli are increased or decreased compared with normal glomeruli or, in other words, whether they are less or more deformable following...
injury is at present unclear. Studies in the 1980s and 1990s suggested that this may be the case (2, 4, 10, 14).

Although cell and tissue mechanical properties change with disease, they are generally believed to be consequences rather than causes of disease. The article in this issue of *American Journal of Physiology-Cell Physiology* by Wyss and colleagues clearly shows how physical factors can act to cause disease (24). In this study, the authors defined the elastic properties of renal glomeruli and identified the cytoskeleton as an important determinant of those properties. In addition, they demonstrated that at an early stage of injury in two genetic mouse models of renal disease, the glomeruli are significantly more deformable than normal even before glomerular structural damage is obvious. Wyss and colleagues used two state-of-the-art techniques, atomic force microscopy (AFM) and “capillary micromechanics” to measure the elastic properties of single glomeruli isolated from healthy and diseased mice (24). Using these two methods, they found that the Young’s modulus (a measure of stiffness) of healthy rat and mouse glomeruli is \(2.5 \text{kPa}\), indicating that a Young’s modulus of \(2.5 \text{kPa}\) may be a general property of mammalian glomeruli. To assess whether the actin cytoskeleton or myosins play a role in determining the elastic properties of glomeruli, the authors treated isolated glomeruli with agents that decrease the levels of polymerized actin (cytochalasin D or latrunculin B) or inhibit the activity of nonmuscle myosins (blebbistatin). Treatment with these three agents led to reduced elastic moduli of the glomerular capillary wall and overall softer glomeruli (1–1.5 \text{kPa}) (24). The authors concluded that glomeruli have defined mechanical properties that depend on cytoskeletal structure, and altering such structure makes glomeruli softer and more deformable.

To determine whether glomerular disease is associated with biomechanical abnormalities and, most importantly, whether these abnormalities contribute to renal disease, the authors used atomic force microscopy (AFM) to compare the Young’s moduli of glomeruli from two mechanistically distinct mouse models of disease, Alport syndrome (mice lacking the \(\alpha 3\) chain of collagen IV), and HIV-associated nephropathy (Tg26HIV/nl mice). At an early stage of disease with minimal pathology at the microscopic level (e.g., glomerular sclerosis), glomeruli from these mice were significantly softer than normal glomeruli with a 30% overall reduction in their Young’s modulus (24). This was an unexpected result as one would predict glomeruli at early stages of disease to be normal and then stiffen with progressive sclerosis. The finding that mechanical changes occur in some models of glomerular disease before morphological changes agrees with the observation that in the liver changes in mechanical properties are evident before the onset of fibrosis, indicating that physical factors may play a primary role in these disease processes (6, 24). The finding that diseased glomeruli are softer than normal glomeruli, agrees with the observations that podocytes isolated from Tg26HIV/nl or \(\alpha 3\)-actinin-4-deficient mice are markedly more deformable than controls, most likely due to disordered cytoskeletons (3, 16, 21). The authors suggest that increased mechanical deformability of glomeruli might be a feature of early states of renal diseases. This alteration could make glomeruli more susceptible to hemodynamic injury with increased distension and thus lead to glomerular cell apoptosis, death, and ultimately glomerular sclerosis (Fig. 1C). Alternatively, this abnormally soft mechanical environment could be inhospitable for differentiated podocytes in a manner similar to that in which

Fig. 1. Changes in the mechanical/elastic properties of glomeruli can lead to renal disease. A: schematic representation of a glomerulus and its major cell components. Mechanical properties (arrows) from both glomerular basement membrane (GBM) and podocyte-derived components contribute to normal glomerular structure and function. B: partial list of proteins that, when mutated or missing from either the extracellular matrix or cell compartments, could lead to changes in the mechanical/elastic properties of the glomerulus, thus leading to renal disease. C: images of glomeruli from a 5-mo-old healthy (left) and Alport (right) mouse. Note the glomerulus of the Alport mouse is severely injured as demonstrated by the presence of glomerular sclerosis (arrows).
normal adherent cells do not grow on soft agar. Conceivably, the early stages of glomerular sclerosis could stimulate an attempt of glomerular cells to reestablish a normal mechanical environment by producing additional matrix and altering its cross-linking (6).

The study of Wyss and colleagues has clinical implications. At present, changes in glomerular morphology (i.e., mesangial expansion; leukocyte infiltration; crescent formation; nodular, segmental, or global fibrosis; GBM abnormalities) together with changes in clinical parameters (i.e., albuminuria, altered glomerular filtration rate, serum creatinine, and other clinical chemistry tests) are used to diagnose renal diseases, establish prognosis, and decide on the appropriate treatment. The study of Wyss and colleagues suggests that although glomeruli might look “normal” with no evidence of morphological and/or clinical changes, they might “hide” a diseased phenotype. The sonographic reading of “increased echogenicity” in diseased kidneys may represent abnormal mechanical properties of kidneys. In other fields such as hepatology and breast disease, elastography (measurement of relative differences in the elasticity of diseased and normal tissues) is being developed as a complement to radiographic and pathologic methods (reviewed in Refs. 12, 17, and 22). To the extent that biophysical differences exist in normal and diseased renal tissue, similar approaches may be beneficial in renal disease. Finally, the findings of Wyss and colleagues are notable in that in contrast to other diseases where greater than normal stiffness is found in disease, reduced stiffness seems to be present in at least some forms of glomerular disease. If this is correct, the pathophysiology of glomerular injury may be different from what is conventionally believed, and interventions directed at tissue mechanics, a mechanism not previously considered, could be important.

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DISCLOSURES

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