

Could Autophagic Exhaustion Be a Final Common Pathway for Podocytopathy in FSGS?

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Among renal syndromes caused by glomerular disorders, FSGS continues to pose the most challenges in understanding its pathogenesis and therefore, in arriving at mechanism-based approaches for its treatment. The extraordinary diversity of genetic etiologies linked to primary FSGS, the sizable number of patients with idiopathic disease, and the disparate clinical conditions unrelated to primary FSGS that manifest the pathology of secondary FSGS make it difficult to formulate a unifying theory of pathogenesis.^{1,2} The common feature that links together diverse genetically determined FSGS, idiopathic FSGS, and other toxic, infective, metabolic, and hemodynamic disorders that cause secondary FSGS is, of course, segmental and global scarring of glomeruli, a pathology now linked to development of podocytopathy.^{1,2} Pathologic features can vary among the gamut of primary and secondary FSGS, but research points to the podocyte as the seat of early as well as continuing responsible pathology. According to evolving dogma, decrease in absolute podocyte numbers primarily, such as in oligomeganephronia, secondarily, through nephron loss by disease, or by cell death during disease is a major factor that fosters progressive glomerular damage. In this line of reasoning, podocytes decrease in number either relatively with respect to the demands of glomerular hypertrophy—such as in remnant kidneys after nephron loss by disease—or by cell death through disease-associated podocytopathy. As a result, remaining podocytes cannot adequately cover the outer surfaces of glomerular capillaries with the differentiated protoplasm required for structural integrity and barrier function.^{3,4} In large part, this is caused by the inability of terminally differentiated podocytes to replenish the population through cell division.⁴ Being possessed of unique structural and biologic attributes that make them vulnerable, surviving podocytes seem unable to adapt and withstand the hemodynamic, metabolic, aging, or disease-related stresses imposed on them or the stresses of pathologic signaling, oxidant injury, misfolded protein responses, cytoskeletal perturbations, or intracellular proteolysis—abnormalities caused by specific

genetic mutations or other disease. If extreme, such stresses cause podocytopathy, cell death, additional podocyte depletion, and secondary endocapillary and mesangial pathologies characteristic of the sclerotic lesion. This pathology progresses segmentally and globally in autonomous vicious cycles: locally within glomeruli, serially injuring and killing adjacent podocytes, and through systemic effects, recruiting more glomeruli to the sclerotic process.^{4–6}

Because the instigating factors that cause secondary FSGS are so disparate and the genetic abnormalities that underlie primary FSGS are so unrelated to each other—affecting proteins related to slit diaphragms, cation channels, mitochondrial proteins, or cytoskeletal proteins—the question arises: is there a common cellular process that connects them, possibly as a final common pathway of cellular degeneration? Apoptosis caused by overactive TGF- β signaling is one possibility,⁷ but there is suggestive evidence to support failed autophagy as another candidate for the putative podocyte pathway to perdition. The two are not mutually exclusive.

Even in normal cells, the wear and tear of life produces misfolded, oxidized, and degraded proteins that aggregate. Organelles become damaged as the result of oxidant stress and endoplasmic reticulum (ER) stress. Autophagy segregates, degrades, and recycles damaged proteins and organelles to maintain cellular health. Of interest, the adult podocyte—like another postmitotic cell: the neuron—displays unusually high rates of autophagic flux, far higher than in tubule cells.^{8,9} Autophagy is not required for the maturation of developing podocytes but is required for healthy maintenance of adult podocytes.^{8,9} In recently reported experiments, autophagy was increased in glomeruli during proteinuria; conversely, podocyte-specific deletion of Atg5—a critical component of the autophagic machinery—impaired the autophagic flux, led to accumulation of oxidized and polyubiquitinated proteins, caused ER stress and proteinuria, and increased the severity of experimentally induced proteinuric disease and age-dependent glomerulosclerosis.^{8,9} These considerations and findings form the basis for an attractive hypothesis—that the podocyte, a high-maintenance cell that normally requires rapid autophagic flux to maintain homeostasis, is susceptible to injury caused by imbalance between increased autophagy requirement and autophagic capacity. Conceivably, such imbalances are caused by disease-induced protein damage and organelle damage. Indeed, it has been proposed that interventions that increase autophagic flux—calorie restriction, mammalian target of rapamycin (mTOR) inhibition by rapamycin, and AMPK activation—could have beneficial effects to retard the progression of glomerulosclerosis.^{10–12} High activity of mTOR inhibits autophagy and could possibly cause autophagic failure and ensuing podocytopathy.^{10–12} Although such a connection seems plausible in diabetic glomerular disease,^{10–12} there is little information regarding mTOR activity as a cause for failed podocyte autophagy in other contexts. Indeed, we know little of how podocytopathy develops in both human disease and experimental models. Nevertheless, major advances in understanding

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the cellular pathology of genetically determined FSGS may provide some clues on which to speculate.

Overexpression of APOL1, mutation of which predisposes for FSGS in African Americans, induces autophagic cell death,¹³ and the protein is depleted in podocytes and proximal tubules in FSGS.¹⁴ These findings raise the questions of whether APOL1 regulates autophagy and whether mutations or deficiency of APOL1 impair autophagy and thereby, cause podocytopathy that leads to FSGS. In a breakthrough of understanding, recent research has uncovered the involvement of cytosolic cathepsin L-mediated proteolysis in the increase of podocyte motility and foot process effacement in proteinuric models.¹⁵ As inferred from interventions involving CD2AP protein and the cation channel TRPC6, both components of the slit diaphragm-podocyte cell junction protein complex, signals emanating from podocyte cell junctions may induce calcineurin-dependent cathepsin L-mediated degradation of critical structural and cytoskeletal proteins in podocytes.^{15,16} This process is assisted by TGF- β -dependent translocation of dendrin from slit diaphragm-cell junction complexes to nuclei and subsequent transcription and expression of cathepsin L.¹⁵ Because mutations of several components of the slit diaphragm-podocyte cell junction complex cause FSGS,^{1,2} it seems possible that cathepsin L-mediated intracellular proteolysis induced by dysfunctional slit diaphragm-podocyte cell junction signaling is a common mechanism for podocyte abnormalities in FSGS. Considering that foot process effacement predates disease-associated podocyte sickness and death, could it be possible that the dysfunctional signaling becomes sustained and eventually, causes uncontrolled proteolysis? In such a scenario, the sick podocyte that is also overworked as a consequence of excessive protein endocytosis may experience autophagic exhaustion. Conceivably, mutations that give rise to mitochondrial damage and cytoskeletal abnormalities—and FSGS—might also result in the accumulation of damaged mitochondria and misfolded/degraded/aggregated proteins—pathologies that need the attention of the autophagic machinery for disposal and recycling. Should the process of protein and/or organelle damage become sustained, the inability of cells to match autophagic needs would result in the accumulation of damaged cellular components. Such imbalance between cellular demand and capacity for autophagy could be projected to secondary FSGS as well in situations where depleted podocytes become stressed. Although speculative, the autophagy hypothesis as a unifying concept deserves our attention. In a sense, this hypothesis was tested recently. Hartleben *et al.*⁸ used podocyte-specific deletion of Atg5 and other interventions to show that podocyte integrity depends on intact autophagic flux; mice with autophagy deficiency in podocytes developed enhanced glomerulosclerosis as they aged (by 20–24 months). Although classic FSGS was not produced, these studies strongly supported autophagic failure as a possible cause for podocytopathy and glomerulosclerosis.

A definitive study linking autophagy failure to FSGS is now reported by Kawakami *et al.*¹⁷ Kawakami *et al.*¹⁷ used Six2

promoter-driven Cre expression to delete Atg5 or -7 from all epithelium derived from metanephric mesenchyme. Atg5 deletion from tubules as well as podocytes produced a disease phenotype with uncanny resemblance to human FSGS by 2–4 months and advanced renal failure by 6 months. Mice developed podocytopathy characterized by foot process effacement and cytoplasmic vacuolization, marked depletion of WT1-positive cells indicative of podocyte depletion, FSGS with hyalinosis, tuft collapse, epithelial proliferation with pseudo-crescent formation, proteinuria, hypoalbuminemia, hyperlipidemia, and renal failure. Notably, there was tubule atrophy and interstitial fibrosis. Atg7 deletion produced a similar but milder phenotype. Protein analysis of kidney extracts showed findings indicative of decreased autophagic flux. Morphologically, podocytes and tubule cells contained decreased numbers of autophagic vacuoles but developed abnormal inclusion bodies. Most importantly, the cells contained swollen, misshapen, and increased numbers of damaged mitochondria and abnormal profiles of ER. These changes were accompanied by features indicative of markedly enhanced oxidant stress and ER stress. The findings are consistent with what we might expect to occur as a consequence of autophagic failure—accumulation of cellular components that become damaged normally and should be recycled by autophagy but are not. Such damaged organelles, particularly mitochondria, may generate increased oxidant species that, if not scavenged adequately, will be cytotoxic. Buttressing this line of thinking, kidneys also showed a deficiency of Mn-SOD, a superoxide scavenger. Kawakami *et al.*¹⁷ propose that tubule abnormalities and podocytopathy have shared roles to produce the FSGS disease phenotype. In support, Kawakami *et al.*¹⁷ offer electron microscopic evidence for mitochondrial abnormalities in biopsies from patients with FSGS. These findings from human kidneys are preliminary, and additional work is needed before such long-reaching extrapolations can be made. It is curious, however, that podocyte-specific Atg5 deletion⁸ did not result in the classic FSGS phenotype that is reported in the work by Kawakami *et al.*¹⁷ Although this could conceivably be explained by incomplete penetrance of podocyte-specific Atg5 deletion in the study by Hartleben *et al.*,⁸ the possibility that a coexisting tubule-based autophagy defect is also necessary to produce the full-blown picture must be investigated. How tubule defects might synergize with podocytopathy to produce the FSGS syndrome is something on which to speculate. Finally, it must be asked if Atg5 deletion during development led to a decrease of nephron number—one factor that could cause FSGS, such as in oligomeganephronia. However, this is probably unlikely, because autophagy is not required for glomerular development.^{8,9}

Overall, the work by Kawakami *et al.*¹⁷ presents novel, important, and fascinating findings that will spur research on the possible role of autophagic failure as a unifying pathology in FSGS. How such autophagic failure might be produced under the numerous and disparate disease circumstances of human and experimental FSGS is easy to speculate on but will be a

formidable question to answer, and it requires a rigorous investigative approach.

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DISCLOSURES

None.

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See related article, "Deficient Autophagy Results in Mitochondrial Dysfunction and FSGS," on pages 1040–1052.

We Don't Prescribe Statins to Lower Cholesterol: We Prescribe Statins to Reduce Vascular Risk

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Statins are an important method for reducing the global burden of noncommunicable chronic diseases (NCDs) such as, vascular disease, hypertension, and diabetes. Statins are one of the most intensively studied medications, with >200,000 patients enrolled in randomized trials. Although statins are not "magic bullets," they have important clinical benefits when used appropriately—including reductions in the risk of death, stroke, and myocardial infarction.

Because higher levels of LDL cholesterol (LDL-C) are associated with excess vascular risk in the general population, it is logical to assume that statins should be prescribed to treat hypercholesterolemia, which in turn reduces the risk of vascular events. This assumption has served patients well,

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