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See related article, “Does AKI Truly Lead to CKD?,” on pages 979–984.

A Photo Shoot of Proteinuria: Zebrafish Models of Inducible Podocyte Damage

Roman-Ulrich Müller*^{†‡} and Thomas Benzing*^{†‡}

*Department 2 of Internal Medicine and Center for Molecular Medicine Cologne, †Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, and ‡Systems Biology of Ageing, University of Cologne, Cologne, Germany

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Diseases of the renal glomerulus, the site of plasma filtration and the production of primary urine, account for a majority of chronic renal diseases.¹ The prevention of ESRD would require therapies that specifically interfere with the pathogenesis of the various underlying glomerular diseases, as well as appropriate models to develop and study targeted interventions.² However, although the last decade has witnessed a breakthrough in our understanding of the pathogenesis of proteinuria and the function of the glomerular filtration barrier, animal models for the study of onset and recovery from glomerular damage are still scarce.

A number of rodent models of inducible podocyte injury have been developed.³ However, these rodent models cannot be used for high-throughput drug screening and have major limitations as a tool for drug discovery aiming at prevention of progressive glomerular damage. In a remarkable paper in this issue of *JASN*, Zhou and Hildebrandt⁴ now present a zebrafish model that allows the induction of glomerular injury and the visualization of a surrogate of proteinuria in real time. The authors used an inducible model of podocyte damage utilizing podocyte-specific expression of prokaryotic nitroreductase. This enzyme converts metronidazol to a cytotoxin. Thus, feeding of metronidazol leads to dose-dependent podocyte loss and glomerular dysfunction.

Podocytes are the visceral epithelial cells of the kidney glomeruli.⁵ Neighboring podocytes extend long, regularly spaced, interdigitated foot processes that enwrap the glomerular capillaries and form a 40-nm-wide filtration slit bridged by a membrane-like cell contact, the slit diaphragm. Together with fenestrated endothelial cells of the glomerular capillaries and the glomerular basement membrane, which separates these two communicating cell types, podocytes form the kidney filtration barrier and restrict the passage of macromolecules on the basis of their size, shape, and charge.^{6,7} Upon podocyte injury, the intercellular junctions and cytoskeletal structure of the foot

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Correspondence: Dr. Thomas Benzing, Department 2 of Internal Medicine, University of Cologne, Kerpener Strasse 62, 50937 Cologne, Germany. Email: thomas.benzing@uk-koeln.de

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processes are altered, and the cell shows an effaced phenotype.⁸ This results in the disappearance of typical slit diaphragm structures and the development of proteinuria. More severe podocyte damage leads to the loss of these cells by apoptosis, necrosis, or detachment, and loss into urine. Because podocytes have a limited capacity for self-renewal, progressive podocyte damage results in glomerulosclerosis, the scarring of the filtration units.^{9,10} Glomerulosclerosis is the basis of progressive renal damage and renal failure requiring renal replacement therapies.

The zebrafish pronephros has been successfully used as a model system for studies on kidney development and function for more than a decade.^{11–16}

Although the pronephric fish kidney differs from the mammalian metanephric kidneys to some extent, the structure of the kidney filtration system is very similar, and the formation of a size-selective filtration barrier in fish required a very similar set of proteins including nephrin, podocin, and slit diaphragm adaptor proteins.¹⁷ Therefore, several investigators have successfully used the fish model system to show that deletion of genes in podocytes resulted in podocyte dysfunction and deficiency of the size-selective kidney barrier.^{17–24}

Over the past several years, zebrafish have emerged as an ideal model to study vertebrate development. The transparency of the zebrafish embryos allows direct visualization of tissue morphogenesis such as glomerular development as it occurs in a live organism. In their study, Zhou and Hildebrandt used fluorescent proteins to visualize glomerular structure without histologic sectioning and were able to determine both podocyte structure and number using confocal microscopy. They found loss of podocytes after induction with metronidazol resulted in severe periorbital and whole-body edema as a result of nephron dysfunction. Activation of caspase-3 indicated that podocytes were lost through apoptosis in this model.

However, an additional intervention takes this novel approach clearly beyond the existing inducible models. The authors simultaneously expressed a transgene coding for fusion of green fluorescent protein to vitamin D binding protein to visualize integrity or dysfunction of the glomerular barrier. This fusion protein mimics the biophysical properties of albumin or other proteins of the circulation and allows for real-time monitoring of proteinuria. Studies into the biology of podocyte function and disease have been hampered by a lack of simple and amendable model systems. Although cultured podocytes and rodent models provide valuable tools in many respects, they are not suited for high-throughput drug screening. Thus, zebrafish being fairly easy to cultivate and showing largely conserved function of many glomerular genes is a promising *in vivo* model.²⁵ Existing zebrafish models of proteinuria rely on the injection of fluorescently labeled dextrans, which is time-consuming and labor-intensive and cannot be used in adult zebrafish.²⁶ Other studies used protein detection in fish water as an indicator of proteinuria. These assays seem to be both fairly cost-intensive, especially when considering large-scale screens, and largely dependent on assay techniques.²³ Using a fluorescent

marker of proteinuria overcomes most of these limitations, which may be interesting in the future.

From the perspective of a nephrologist, screening for novel drugs that prevent podocyte death and the development of progressive glomerulosclerosis would be most desirable.²⁷ Of note, zebrafish embryos and larvae have successfully been used in high-throughput drug discovery screens targeting polycystic kidney disease.^{13,28} Moreover, it may be possible that quantitative measurement of proteinuria in fish through quantification of green fluorescent protein in the water of the living animal using ELISA kits may be possible in the future. Therefore, combining visualization of changes in glomerular structure with the measurement of proteinuria may be a very powerful technology for screening into novel therapeutic agents.

Another aspect of this interesting work is that the authors went on to show that loss of podocytes leads to the expression of the zebrafish-specific *WT1* homolog, *wt1b*, in cells surrounding Bowman's capsule. *Wt1b* has been shown previously to be a marker of kidney regeneration in zebrafish.²⁹ The limited podocyte self-renewal in vertebrates seems to be mediated by a cellular niche of similar localization. Whether this induction of *Wt1b* is indeed part of a mechanism of glomerular repair through podocyte renewal cannot be answered from their studies. However, this zebrafish model provides the opportunity to address this question in the future.

It is worth noting that this paper has the same limitations as many publications on model systems using artificial mechanisms of podocyte damage that are considerably different from the mechanisms involved in human disease. Despite this limitation, Zhou and Hildebrandt have undoubtedly provided an interesting tool for future studies into the mechanisms and, more importantly, the prevention of podocyte loss and progressive glomerular disease.

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DISCLOSURES

None.

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See related article, “Inducible Podocyte Injury and Proteinuria in Transgenic Zebrafish,” on pages 1039–1047.

Human AKI and Heme Oxygenase-1

Karl A. Nath

Division of Nephrology and Hypertension, Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota

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Findings derived from animal models of AKI gain clinical credence and appeal when translational studies corroborate their occurrence in human AKI. In the current issue of *JASN*, Zager *et al.*¹ take important steps in addressing the clinical applicability of the conclusion drawn from animal models that induction of heme oxygenase-1 (HO-1) is a protective response in AKI.

The major heme-degrading mechanism in tissues, heme oxygenase has its origin in studies probing the metabolic fate of hemoglobin during the culling of senescent erythrocytes by the reticuloendothelial system.² It was then realized that there were two isoforms with heme oxygenase activity^{3,4}: the constitutive isoform, HO-2, and the inducible isoform, HO-1; the latter was identified as a cytoprotective gene in a model of AKI induced by heme proteins.⁵ Subsequently, induction of HO-1 was recognized as a protective response that can occur in all organs and tissues and against virtually any insult.^{3,4,6–10}

At least four features of HO-1 underlie its salutary effects in stressed tissues.^{11,12} First, HO activity generates three chemically distinct products—carbon monoxide, bile pigments, and iron—each of which can participate in specific cellular processes; induced HO-1 thus transmits broad-based and far-ranging signals through its tripartite products and their respective downstream effects. Second, all products of HO activity are involved in a number of homeostatic mechanisms; induced HO-1 thus readily communicates with networks that influence cell survival. Third, ischemic and toxic insults can destabilize intracellular heme proteins (for example, cytochrome p450), and heme that is released can itself perpetrate cell injury; induced HO-1 thus vitiates secondary

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Correspondence: Dr. Karl A. Nath, Mayo Clinic, Guggenheim 542, 200 First Street SW, Rochester, MN 55905. Email: nath.karl@mayo.edu

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