2015 Homer W. Smith Award: The Podocyte from Periphery to Center Stage

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ABSTRACT
This overview summarizes selected major developments over the last 40 years in understanding podocyte biology and its involvement in glomerular disease subjectively from my perspective. Serendipity has played a major role in my contributions to investigative nephrology that range from basic mechanisms of immune deposit formation in experimental membranous nephropathy to the role of a microRNA in FSGS. This review emphasizes the importance of continuous reality checks of experimental results obtained in vitro or with genetically modified animals with human disease.

It is a great honor, especially for a pathologist, to receive the Homer W. Smith Award of the American Society of Nephrology. I humbly accept this prestigious prize in lieu of my fellow renal pathologists who contribute with their diagnostic biopsy service to the clinical management of patients. On top of this everyday business, pathologists are dedicated and strong partners in investigative nephrology who help to unravel the causes of renal diseases that are the basis for targeted treatments.

Determining one’s current position in science and life in general and avoiding idle rediscoveries sometimes are helped and inspired by a glance back into the past to find out which ideas and concepts persisted and could be of use in current thinking. Thus, taking an example from double-faced Janus, the Roman God of the Beginning and the End, I will look into the past to identify what have we learned in the last decades that is still valid and helpful to understand the mechanisms of glomerular disease today. Also, I will try to look into the future and discuss a novel development that could become potentially important in understanding and managing glomerular diseases.

PODOCYTES MOVE CENTER STAGE

Given the beauty of the architecture and the functional importance of podocytes, it is surprising that they became a hot topic in research only in the late 1980s. Before this time, the focus of investigation on tubular function presumably was because of the long shadow cast by the scientific genius of Homer W. Smith, who arguably paved the way for renal research as we know it today. Podocyte research became only slowly popular by the early pioneering work of Harrison Latta1 at the University of California, Los Angeles, Marilyn Farquhar2,3 and Robert Venier2 at Minneapolis and Rockefeller, respectively, and Morris Karnovsky4 at Harvard. The field received a strong boost from the availability of cultured podocytes from the laboratory of Wilhelm Kriz and Peter Mundel5 in Heidelberg and the discovery of nephrin by Karl Tryggvason6 in Stockholm to mention only two outstanding landmarks.

FROM RAT HEYMANN NEPHRITIS TO HUMAN MEMBRANOUS NEPHROPATHY

Pathologists have the privilege of physically seeing typical patterns of tissue damage in biopsies through the microscope, and this morphologic approach provided the basis of classification of glomerular and other diseases that remains today. The stereotypic histopathologic changes in a given disease continue to stimulate consideration of its molecular and cellular bases, and this is particularly true for membranous nephropathy. This disease is caused by formation of immune deposits composed primarily of IgG4 that are arranged in specific subepithelial localization in the lamina rara externa of the glomerular basement membrane.

THE AUTOANTIGEN OF MEMBRANOUS NEPHROPATHY

What are the mechanisms of immune deposit formation, and what can we learn about pathways leading to glomerular damage in membranous nephropathy?
Such questions are impossible to address without animal models. Fortunately, a reproducible, robust model of membranous nephropathy in rats was coincidently discovered by Walter Heymann in Cleveland, Ohio—the disease that still carries his name. The initial concepts came from Frank Dixon and Richard Glasscock at the Scripps Foundation in La Jolla, California, who proposed that immune complexes consisting of a then unidentified “Heymann auto-antigen” within the renal tissue and autoantigen–specific IgG form in the circulation and are deposited as granules in the glomerular basement membrane. This view dominated the field for more than two decades but was eventually challenged by William Couser’s group (then in Boston, MA) and Philipp Hoedemaeker and coworkers in Leiden, The Netherlands. They provided compelling evidence that the antigen target(s) must be located somewhere within the glomerulus, because perfusion of isolated rat kidneys with pathogenic antibodies rapidly generated typical immune deposits in situ in the absence of preformed immune complexes. These results raised the questions of the identity and localization of the pathogenic antigen(s) within the glomerulus. Several groups participated in this quest, including those of Pierre Ronco and Pierre Verroust in Paris, France, Aaro Miettinen in Helsinki, Finland, and Sudesh Makker in Little Rock, Arkansas.

At this point, I take the liberty to include a few words on my personal history. I was accepted as a postdoctoral fellow in 1980 into the laboratory of Marilyn Farquhar, then in the Section of Cell Biology at the Yale School of Medicine directed by the Nobel Prize–winning cell biologist George Palade. Using the then novel method of immunoprecipitation with nephrogenic sera (that I had brought with me from Vienna, Austria) and isolated renal proximal tubule brush border (that I learned to prepare from Peter Aronson in the Yale Physiology Department), it became possible to identify and isolate the antigen. It turned out to be a very large molecule, and in the absence of reliable molecular markers in those days, we estimated its molecular mass at 330 kDa, which in retrospect, was definitely wrong. The group of Sudesh Makker later came up with the correct molecular mass, which was about double our estimate, and therefore, we subsequently called the molecule megalin to suggest its large size. We found that megalin is expressed in clathrin-coated pits, suggesting even at this early stage that we might be dealing with a receptor. Later, Akira Saito in the Farquhar laboratory cloned and manually sequenced megalin and established that it is a member of the LDL receptor family, similar to the later described polyspecific receptor LRP-1.

In retrospect, the major finding that has directed the thinking of several investigators in the nephrology field ever since was that the membrane glycoprotein megalin is expressed exclusively in podocytes and nowhere else in the glomerulus. This turned the spotlight on the podocyte as a crucial presenter of antigens in situ and a source of immune deposits and presumably, also glomerular damage, at least in rat Heymann nephritis. However, it came as a disappointment that megalin was obviously irrelevant for human membranous nephropathy, because it is not expressed by human podocytes. Despite this, the principle that membranous nephropathy is caused by the autoimmune response to antigens of the podocyte surface remains correct.

Recently, three landmark reports have identified the relevant glomerular antigens in the human glomerulus, coming from the laboratories of Pierre Ronco in Paris, France (dipeptidyl peptidase in a hereditary setting), David Salant and Lawrence Beck in Boston, Massachusetts (phospholipase A2 receptor), and Rolf Stahl in Hamburg, Germany (thrombospondin type 1 domain–containing 7A). These antigens account for most, if not all, cases of primary human membranous nephropathy. Intriguingly, all identified antigens have in common with megalin that they are associated with and displayed by the cell membrane of podocytes. Taken together, this shows that the in situ targets for different pathogenic antibodies in human membranous nephropathy and rat Heymann nephritis are localized in the podocyte cell membrane and presumably, variations on a common mechanistic and pathogenic in situ theme.

MECHANISMS OF GLOMERULAR INJURY IN MEMBRANOUS NEPHROPATHY

Proteinuria is the clinical hallmark of both rat Heymann nephritis and human membranous nephropathy. Again, studies on Heymann nephritis have helped us to understand how glomerular damage and proteinuria in membranous nephropathy actually occur. Results primarily from William Couser’s group (then in Seattle, WA) have established that the complement system plays an important pathogenic role in proteinuria. In particular, the C5b–9 membrane attack complex was inserted into the basolateral podocyte cell membrane that attaches to the basal membrane. This causes podocytes to overexpress the NADPH oxidoreductase complex in large amounts, triggering the formation and release of oxygen radicals that diffuse into the glomerular basement membrane. This by itself could cause aggregation and degradation of major matrix proteins, at least in vitro, and disrupt the basement membrane’s texture.

However, another potentiation of the damaging effects of free radicals was achieved by a functional property of megalin itself. Work by Eric Christensen in Aarhus, Denmark, Pierre Verroust in Paris, France, and Thomas Willnow in Berlin, Germany showed clearly that megalin is a polyspecific receptor. In the context of glomerular injury, apoB and apoE are important, because megalin serves as a molecular uptake device for lipid-rich lipoproteins into rat podocytes. However, the immune deposit forming antimegalin IgG is directed against exactly the same ligand binding sites on megalin, blocks the internalization of lipoproteins, and causes their massive accumulation within immune deposits. The in situ collision between reactive oxygen species and lipids enhanced glomerular injury primarily by
lipid peroxidation that generates scores of highly toxic compounds, such as dialdehydes. These crosslinking agents form covalent adducts with matrix proteins, in particular with NC1 domains of type 4 collagens, and result in distortion of the matrix texture of the glomerular basement membrane and proteinuria. The potential clinical relevance of these findings was supported by therapeutic studies with the lipid peroxidation scavenger probucol that decreases proteinuria in both Heymann nephritis and human membranous nephropathy, thus suggesting similar mechanisms in both human and experimental rat membranous nephropathy.

**TRANSLATIONAL INSIGHTS**

Taken together, the analysis of the pathogenic mechanisms of rat Heymann nephritis has highlighted the role of podocytes in a dual role (i.e., by providing the membrane protein targets for pathogenic antibodies and delineating the molecular course of events, leading to breakdown of the glomerular filtration barrier). These insights obtained in the experimental rat model have provided the scaffold for the investigations on human membranous nephropathy and revealed in essence themes with variations in the pathogenic events in patients.

**NOVEL PATHOGENIC MECHANISMS OF FSGS**

In the second part of my presentation, I will look into the future that started for us with a study of FSGS that is considered a podocytopathy. In this disease, mature podocytes are flattened—as in their early development—and the filtration barrier becomes very leaky. The glomeruli react to these changes with segmental, focal sclerotic lesions, hence the descriptive name. This has raised a fundamental question: how do mature podocytes keep their shape? Over the years, many structural proteins have been discovered that are directly responsible for maintaining this complex structure; examples include nephrin, CD2AP, and podocin of the slit diaphragm domain and several others as summarized in recent comprehensive reviews. More recently, regulatory molecules and systems have also been identified that indirectly support the podocyte’s architecture. These include microRNAs (miRs) that are, in essence, cellular tools to fine tune gene expression in a rapid but precise fashion.

**MIR193A REGULATES THE PODOCYTE’S MASTER GENE WILMS TUMOR 1**

Our story started with a serendipitous observation. Gene array data of breast cancer cells grown in vitro have shown that mir193a correlates with the metastatic potential of tumor cell clones, and a mouse carrying an inducible transgene for this miR was generated. However, these mice never develop tumors, because they die too early: within 40 days of the introduction of doxycycline feeding with the homozygous animals and around 70 days for the heterozygotes. Intriguingly, the cause of death was contracted end stage kidneys. In situ hybridization indicated that mir193a was overexpressed primarily in podocytes for reasons that we still do not understand. Analysis of the time course of the renal disease showed that we had actually generated a new reproducible model of FSGS. Typically, this started with small foci of sclerosis after 14 days of induction and evolved over around 70 days to global glomerular scarring. With this information, it was easy to discover the targets of mir193a within podocytes. We identified about 200 targets by deep sequencing of the RNA of isolated glomeruli, and one stood out, namely the Wilms tumor 1 (WT1) gene, that is considered a master regulatory gene of early podocyte development but whose function was not that clear in adults. Expression of WT1 was dramatically reduced by overexpression of mir193a as well as other genes downstream that rely on the function of WT1. This list comprised essentially all proteins that have been involved in the flattening of foot processes.

**MIR193A IN HUMAN FSGS**

Was the glomerular disease that we had produced by overexpression of mir193a just another experimental mouse disease, or was it relevant to human FSGS? To find out this, we analyzed archived paraffin sections of renal biopsies from different glomerular diseases, including IgA, membranous glomerulopathy, minimal change disease, and FSGS. Glomeruli were microdissected from paraffin sections for determination of the expression levels of mir193a. We found that mir193a was selectively overexpressed in about 70% of cases with FSGS but was not overexpressed in other glomerular diseases. There were few cases with very high expression, among them HIV infections, whereas in the few cases available with proven genetic cause of FSGS, mir193 was expressed in the same low levels found in normal controls, presumably because the established genetic defects hit structural podocyte proteins directly without need for mir193a for dysregulation. A more detailed analysis of the expression of mir193a in subgroups of FSGS is required and currently in progress.

**TARGETED THERAPY WITH A SPECIFIC LOCKED NUCLEIC ACID**

Can we influence the development of mir193a-induced FSGS with a specific, undegradable, synthetic, locked nucleic acid (LNA) that is directed against the binding site of mir193a? Initially, we were disappointed that all of the widely used standard rodent FSGS models failed to overexpress mir193a, suggesting that their pathogenesis was different from that of our mir system and presumably, that of human FSGS. Therefore, we intraperitoneally injected the mir193a-specific nano–LNA or a control scrambled LNA during induction of mir103a by doxycycline feeding and found that the glomerular pathology
was dramatically reduced along with the proteinuria.\textsuperscript{35} We take these experiments as initial proof of principle for a targeted specific therapy of an miR–induced glomerular disease.

**MIR193A AND GLOMERULAR CAPSULAR CRESCENTS**

Initially, we localized miR193a in normal human glomeruli by \textit{in situ} hybridization, and we found only traces in podocytes, as expected; in contrast, there was massive expression in the parietal epithelial cells of Bowman’s capsule. At this point, Catherine Meier-Schwesinger from Hamburg, Germany joined forces with us. She had cultured parietal epithelial cells that were microdissected from isolated human glomeruli and expressed several of the key markers of this cell type, such as Pax-8, as well as relatively high amounts of miR193a. However, when we selectively knocked down this miR, the cells started to express typical podocyte markers, such as α-actinin. This has raised the possibility that miR193a could control the transformation of parietal to visceral glomerular epithelial cells.\textsuperscript{36} In accordance with this view, we found that podocytes in early renal development massively express miR193a, which fades away as the podocytes mature. The finding that miR193a was concentrated in parietal epithelial cells raised the possibility that it could also be expressed in capsular crescents. This was, indeed, the case in both humans and mice with antiglomerular basement membrane disease, in whom the growth of crescents was significantly reduced by miR193a-specific LNA but not scrambled controls.\textsuperscript{36}

**ACKNOWLEDGMENTS**

All of the work presented here as well as several other projects in the laboratory would not have been possible without an outstanding faculty in Vienna and a dedicated expert technical staff. I am especially grateful to my fellow nephropathologists Dr. Renate Kain, who is working on ANCA–related crescentic glomerulopathy, and Heinz Regele, who has contributed to the understanding of humoral transplant rejection. We were also blessed by outstanding visiting faculty: Dr. James Neale from Christchurch, who did most of the work on oxygen radicals and passed away too early; Dr. Robert Atkins from Melbourne; Dr. Katsuyuki Matsui from Tokyo, who made mAbs against several glomerular antigens, including a glycoprotein that we have named podoplanin that was the first stable marker for lymphatic endothelial cells; and Dr. Andrew Rees from Aberdeen, who is a permanent source of inspiration. Finally, I have to apologize to all colleagues whose important work was not cited in this short overview because of shortage of space.

**DISCLOSURES**

None.

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**PERSPECTIVE ON MIRS CONTROLLING PODOCYTES**

Taken together, these results then suggest that miR193 could be important for podocyte structure and stability of the glomerular barrier function and that an LNA could antagonize overshooting miR193a production \textit{in vivo}. Is this a proof of principle? Could miRs in podocytes be potential therapeutic agents? It is not clear yet how many miRs are necessary for maintaining a healthy podocyte, but the fact that a single LNA specific for miR193a can antagonize so much of glomerular pathology shows that this miR seems to have a major role in the pathology in both experimental mouse disease and presumably, humans as well.

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