Founding papers of current nephrology: from acute kidney injury to diabetic kidney disease

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KEYWORDS: acute kidney injury; biomarker; diabetic kidney disease; erythropoietin; hyperfiltration; hypoxia

Kidney International was fortunate to publish the 5 seminal papers summarized below. The first 3 studies induced a paradigm shift in understanding of the pathophysiology and clinical practice of acute kidney injury and diabetic kidney disease. The last 2 highlight the importance for nephrology research of new tools such as differentiated cell lines and transgenic animals for the identification of erythropoietin-producing kidney cells.

Glomerular hemodynamics in experimental diabetes mellitus

Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int. 1981;19:410–415. The glomerular hyperfiltration theory proposed by Barry Brenner explains that glomerular hypertension mediates progressive renal damage after any of a variety of initiating injuries. Glomerular hyperfiltration can be caused by afferent arteriolar vasodilation as seen in patients with diabetes or after a high-protein diet, and/or by efferent arteriolar vasoconstriction owing to activation of the renin–angiotensin system, thus leading to glomerular hypertension. The various diseases that have been associated with glomerular hyperfiltration include diabetes mellitus, secondary focal segmental glomerulosclerosis caused by a reduction in renal mass, and obesity-related glomerulopathy. This theory created a paradigm shift in nephrology,[1] had a huge impact on research and clinical practice in nephrology, and provided a theoretical background to the therapeutic approach of blocking the renin–angiotensin system.

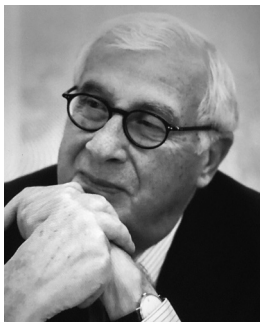

Figure 1 |

At the beginning of the1980s, Brenner and colleagues proposed that maladaptive glomerular hemodynamic changes exert a major influence on the factors that initiate and perpetuate kidney disease progression. In 1981, Hostetter, Troy, and Brenner performed micropuncture studies in control and hyperglycemic Munich-Wistar rats.[2] In this milestone paper that we selected from among many others, they found glomerular hyperfiltration in moderately hyperglycemic rats. The alterations in single-nephron glomerular filtration rate in hyperglycemic rats mimicked the changes in glomerular filtration rate observed in diabetic patients with analogous degrees of hyperglycemia. Based on this seminal work, they speculated that "It is possible that one or more hormonal systems are responsible for the observed resetting of the glomerular pressures and flows in diabetic rats. . . . Of note, the diabetic state has also been shown to be associated with alterations in vascular responsiveness to such potent vasoactive substances as angiotensin II and catecholamines, humoral agents capable of initiating profound alterations in glomerular microcirculatory dynamics." In the same year, they showed that hyperfiltration can be a mediator of progression of kidney disease in the remnant kidney model.[3]

These groundbreaking works have transformed our practice. Today, we use angiotensin-converting enzyme inhibitors and angiotensin receptor blockers to reduce glomerular hyperfiltration as the first choice in patients with diabetic kidney disease and other proteinuric kidney diseases, as large-scale

Masaomi Nangaku[1]
[1]*Division of Nephrology and Endocrinology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan*

Correspondence: *Masaomi Nangaku, Division of Nephrology and Endocrinology, The University of Tokyo Graduate School of Medicine 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: mnangaku-tky@umin.ac.jp*

Editor's Note

This article is part of the *Kidney International* 60th anniversary series. This month's topics cover acute kidney injury, diabetic kidney disease, and cell biology.

clinical trials demonstrated kidney-protective effects of these reagents. Hyperfiltration can also be the target of the powerful kidney-protective effects proven by recent clinical trials of SGLT2 inhibitors.[4]

Figure 1 shows Barry M. Brenner, Director Emeritus, Renal Division, Brigham and Women's Hospital, and Distinguished Levine Professor of Medicine at Harvard Medical School (courtesy of Barry Brenner). Reprinted with permission from Giebisch GH. A long affair with renal tubules. *Annu Rev Physiol* 2011;73:1–28. Copyright © 2011 Annual Reviews. All rights reserved.

Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial

The Diabetes Control and Complications Trial (DCCT) Research Group. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. Kidney Int. 1995;47:1703–1720. The Diabetes Control and Complications Trial (DCCT) is a landmark, multicenter, randomized, clinical trial designed to determine whether an intensive treatment regimen directed at maintaining blood glucose concentrations at levels as close as possible to normal values will affect the development or progression of complications in patients with insulin-dependent diabetes mellitus. This is the longest and largest prospective study showing beneficial effects of intensively lowering blood glucose concentration on diabetic complications.

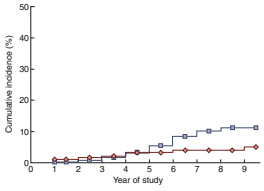

Figure 2|

In the milestone paper published in 1995, the DCC Research Group presented a detailed description of the effects of this treatment on diabetic nephropathy.[5] In the primary prevention cohort (no complications of diabetes at baseline), intensive treatment reduced the mean adjusted risk of the cumulative incidence of microalbuminuria by 34%. In the secondary intervention cohort with early complications of diabetes at baseline, including albumin excretion rate <139 micrograms/min, intensive therapy reduced the mean adjusted risk of microalbuminuria by 43%, the risk of a more advanced level of microalbuminuria (>70 micrograms/min) by 56%, and the risk of clinical albuminuria (>208 micrograms/min) by 56% (Figure 2).

The results of the DCCT provided much support for intensive treatment to reduce the development of diabetic complications such as diabetic kidney disease. Particularly encouraging has been the continuing difference between the 2 groups, despite their having similar diabetic control levels since the end of DCCT, when most of participants entered the observational Epidemiology of Diabetes Interventions and Complications (EDIC) study.[6] This continuing relative benefit has been called a "legacy effect" and "metabolic memory." Potential mechanisms of metabolic memory have been a focus of intensive research and may be multifactorial, including the Maillard reaction and epigenetic changes.[7]

Figure 2 shows the cumulative incidence of development of clinical albuminuria among all subjects in the intensive (diamonds) and conventional (square) treatment groups in the secondary prevention cohort. Adapted from The Diabetes Control and Complications Trial (DCCT) Research Group. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. *Kidney Int.* 1995;47:1703–1720.[5] Copyright © 1995, International Society of Nephrology.

Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury

Han WK, Bailly V, Abichandani R, et al. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int. 2002;62:237–244. Early diagnosis is key to earlier intervention and improving outcomes of patients with acute kidney injury. Not surprisingly, the quest for a sensitive and specific biomarker that could allow early detection of acute kidney injuryhas been intense. In 2002, in this journal, Joe Bonventre's group first described kidney injury molecule-1 (KIM-1), which has proved to be a good biomarker for acute kidney injury. KIM-1 is a type 1 transmembrane protein, whose expression is markedly upregulated in the proximal tubule in the post-ischemic rat

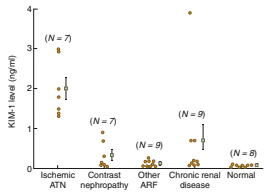

Figure 3|

kidney.8 The ectodomain of KIM-1 is shed from cells. Bonventre and collaborators evaluated kidney tissue samples from patients with biopsy-proven acute tubular necrosis by immunohistochemistry for expression of KIM-1. They also analyzed urine samples from patients with various acute and chronic kidney diseases, as well as from normal controls. They found extensive expression of KIM-1 in proximal tubule cells in biopsies from all the patients with confirmed acute tubular necrosis. The normalized urinary KIM-1 levels were significantly higher in patients with ischemic acute tubular necrosis compared to levels in patients with other forms of acute renal failure or chronic kidney disease (Figure 3). Adjusted for age, gender, length of time delay between the initial insult and sampling of the urine, a one-unit increase in normalized KIM-1 was associated with a greater than 12-fold risk for the presence of acute tubular necrosis, whereas concentrations of other urinary biomarkers did not correlate with clinical diagnostic groupings.

Since then, utility of KIM-1 as a biomarker in humans has been confirmed, and it is accepted by the US Food and Drug Administration and the European Medicines Agency as a highly sensitive and specific urinary biomarker to monitor drug-induced kidney injury in preclinical studies and on a case-by-case basis in clinical trials. Experimental studies suggest that KIM-1 expression is protective during early injury, whereas in chronic disease states, prolonged KIM-1 expression may be maladaptive and may represent a target for therapy of chronic kidney disease.

Figure 3 shows a comparison of urinary KIM-1 concentration in various forms of kidney diseases. Scattergram of absolute KIM-1 concentration is shown to discriminate the value of KIM-1 among the different groups of patients. Adapted from Han WK, Bailly V, Abichandani R, et al. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int.* 2002;62:237–244.[8] Copyright © 2002, International Society of Nephrology.

HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney

Ryan MJ, Johnson G, Kirk J, et al. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. Kidney Int. 1994;45:48–57. *In vitro* studies contribute a great deal to the understanding of biology and pathophysiology. Primary cultured cells are isolated directly from tissues, and Gary Striker[9] was the first to successfully grow glomerular cells in culture in 1970. Although primary cultured cells may be more physiological, they have a finite lifespan and limited expansion capacity. In contrast, cell lines are generally more robust and easier to work with. They proliferate rapidly and have unlimited growth potential. Therefore, it is ideal if a cell line that retains physiological characteristics of its *in vivo* cell counterpart is available.

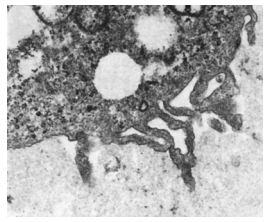

Figure 4|

In 1994, Ryan and colleagues established a human proximal tubular cell line by transduction with human papilloma virus (HPV 16) E6/E7 genes.[10] They obtained a primary proximal tubular cell culture from normal human kidney cortex. Organs were procured for transplantation from a noninfectious adult male, and one kidney, deemed unsuitable for transplantation due to a cortical scar, was released for this research purpose. Exposure to a recombinant retrovirus containing the HPV 16 E6/E7 genes allowed them to obtain a cell line designated HK-2 (human kidney-2). HK-2 cells grow continuously in serum-free media and retain a phenotype indicative of well-differentiated proximal tubular cells, including positivity for alkaline phosphatase and gamma glutamyl transpeptidase and negativity for markers of other cell types (Figure 4). Furthermore, HK-2 cells retain functional characteristics of proximal tubular epithelium. HK-2 cells showed Na+-dependent/phlorizin-sensitive sugar transport and adenylate cyclase responsiveness to parathyroid, but not to antidiuretic hormone.

This new proximal tubular cell line has had substantial research application since then and continues to contribute enormously to nephrology research even today.[11] A PubMed search with the key word "HK-2" returned more than 1900 research articles in mid-March 2020.

Figure 4 shows electron microscopy of HK-2 cells demonstrating microvilli reminiscent of brush border. Adapted from Ryan MJ, Johnson G, Kirk J, et al. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. *Kidney Int.* 1994;45:48–

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Identification of the renal erythropoietin-producing cells using transgenic mice

Maxwell PH, Osmond MK, Pugh CW, et al. Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int. 1993;44:1149–1162. Erythropoietin is a hormone secreted by the kidney in response to cellular hypoxia, which stimulates red blood cell production in the bone marrow. Anemia is a common complication of chronic kidney disease and is mainly caused by the inability of injured kidneys to produce appropriate amounts of erythropoietin. However, the cell type that produces erythropoietin in the kidney remained unknown for a long time. The proposed candidates included juxtaglomerular apparatus, mesangial cells, endothelial cells, and interstitial cells.

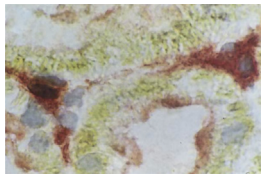

Figure 5 |

Sir Peter Ratcliffe and his colleagues attacked uncertainty about the identity of the kidney cells involved in erythropoietin production.[12] They used sequence from the mouse erythropoietin locus to direct expression of a marker gene, SV40 antigen, to these cells in transgenic mice. In transgenic mice bearing SV40 sequence at homologous and heterologous insertion sites, expression of the marker gene was restricted to a population of cells in the interstitium of the cortex and outer medulla (Figure 5). Immunohistochemical characterization by light and electron microscopy showed that these are the fibroblast-like type I interstitial cells. This study clearly concluded that erythropoietin-producing cells in the kidney are fibroblast-like interstitial cells.

And the rest is history, and *Kidney International* is proud to be part of it. Sir Peter Ratcliffe extended his studies and eventually elucidated the mechanism of a widespread cellular oxygen sensor, that is, hypoxia-inducible factor (HIF) and prolyl hydroxylase.[13] Now prolyl hydroxylase inhibitors that activate HIF are clinically used at the bedside as a novel therapeutic modality for the anemia of chronic kidney disease.[14] Sir Peter Ratcliffe became the first nephrologist awarded the Nobel Prize in Medicine or Physiology, for his seminal work with 2 other oxygen biologists, William G. Kaelin and Gregg Semenza, in 2019.

Figure 5 shows double staining for SV40T Ag and 5' NT. Two interstitial cells positive for 5' NT are seen (red staining) lying adjacent to a proximal tubule. One of these is also positive for SV4O T Ag (black nucleus). Several other interstitial cells are negative for both markers. Adapted from Maxwell PH, Osmond MK, Pugh CW, et al. Identification of the renal erythropoietin-producing cells using transgenic mice. *Kidney Int.* 1993;44:1149–1162.[12] Copyright © 1993, International Society of Nephrology.

REFERENCES

1. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int*. 1996;49:1774–1777.
2. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int*. 1981;19:410–415.
3. Hostetter TH, Olson JL, Rennke HG, et al. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol*. 1981;241:F85–F93.
4. Heerspink HJL, Kosiborod M, Inzucchi SE, Cherney DZI. Renoprotective effects of sodium-glucose cotransporter-2 inhibitors. *Kidney Int*. 2018;94:26–39.
5. The Diabetes Control and Complications Trial (DCCT) Research Group. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. *Kidney Int*. 1995;47:1703–1720.
6. DCCT/EDIC Research Group. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. *N Engl J Med*. 2011;365:2366–2376.
7. Hirakawa Y, Tanaka T, Nangaku M. Mechanisms of metabolic memory and renal hypoxia as a therapeutic target in diabetic kidney disease. *J Diabetes Investig*. 2017;8:261–271.
8. Han WK, Bailly V, Abichandani R, et al. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int*. 2002;62:237–244.
9. Quadracci L, Striker GE. Growth and maintenance of glomerular cells in vitro. *Proc Soc Exp Biol Med*. 1970;135:947–950.
10. Ryan MJ, Johnson G, Kirk J, et al. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. *Kidney Int*. 1994;45:48–57.
11. Jao TM, Nangaku M, Wu CH, et al. ATF6α downregulation of PPARα promotes lipotoxicity-induced tubulointerstitial fibrosis. *Kidney Int*. 2019;95:577–589.
12. Maxwell PH, Osmond MK, Pugh CW, et al. Identification of the renal erythropoietin-producing cells using transgenic mice. *Kidney Int*. 1993;44:1149–1162.
13. Eckardt KU. The noblesse of kidney physiology. *Kidney Int*. 2019;96:1250–1253.
14. Sugahara M, Tanaka T, Nangaku M. Prolyl hydroxylase domain inhibitors as a novel therapeutic approach against anemia in chronic kidney disease. *Kidney Int*. 2017;92:306–312.