Applying proteomics to detect early signs of chronic kidney disease: where has the magic gone?

Jon B. Klein
Division of Nephrology and Hypertension, University of Louisville School of Medicine, Louisville, KY, USA

ARTICLE HISTORY Received 5 January 2017; accepted 31 March 2017

KEYWORDS Biomarkers; chronic kidney disease; diabetes; glomerulonephritis; peptidomics

1. Introduction

In 2003, the NIH Director Elias Zerhouni published his description of the NIH Roadmap, a series of initiatives that were designed to accelerate the pace of medical research [1]. Particularly emphasized in the Roadmap was the development of ‘New Pathways to Discovery’ that included initiatives in proteomics. The Roadmap initiatives also placed an emphasis on ‘technologies that improve assessment of clinical outcomes,’ thereby igniting interest in biomarker development using genomic and proteomic tools. The US FDA Critical Path Initiative (CPI) that was intended to modernize drug discovery followed the NIH Roadmap in 2004. The development of new biomarkers was the highest scientific priority of the CPI, and the influence of the FDA undoubtedly spurred even more interest in biomarker discovery and validation [2]. A number of research organizations, sensing the new directions (and funding), set down by the Roadmap and the CPI, convened consensus conferences to lay out their own research priorities. In the renal research community, the American Society of Nephrology conducted a series of research retreats with experts in the fields of acute renal failure, diabetic nephropathy, hypertension, transplantation, and uremic cardiovascular toxicity. Participants in the retreat included clinicians and scientists with a background in renal research, as well as representatives from National Kidney Foundation, the American Society of Pediatric Nephrology, the American Society of Transplantation, the American Society of Hypertension, and the Kidney Council of the American Heart Association, the NIH and the FDA. The summary of these retreats [3] placed a strong emphasis on the discovery of biomarkers of renal disease and set the highest priority on finding biomarkers to:

- Diagnose acute kidney injury (AKI) before a rise in serum creatinine
- Stratify patients with respect to severity of (renal) injury
- Provide prognostic indicators.

With the Roadmap, the CPI, and the American Society of Nephrology Renal Research Report, the stage was set by 2004 for a vigorous effort to find new biomarkers of renal disease. Because of the clinical need and preexisting biomarker targets, a good deal of the renal biomarker research initially focused on AKI. The path to AKI biomarker validation has been arduous and has recently been reviewed [4], and the development of chronic kidney disease (CKD) biomarkers has faced many of the same barriers as AKI biomarker discovery. First and foremost is the barrier imposed by the use of creatinine as the ‘gold standard’ of renal biomarkers. The measurement of serum creatinine has been used as a surrogate marker of renal function since 1926 when Poul Rehberg determined that creatinine reflected renal function and not hepatic function as had been believed previously [5]. While the serum creatinine is often referred to as the ‘gold standard,’ it suffers from many flaws as a measurement of renal function and CKD and is merely the most commonly used measure of renal function. Some of these flaws include an altered half-life when renal function falls below 50% of normal, substantial renal reserves that are not reflected in serum creatinine levels, and marked variability caused by weight, sex, drugs, and protein metabolism. Based on these limitations, the renal research community launched a number of initiatives to find a biomarker that could supplant creatinine as the ‘gold standard’ of renal function measurement.

2. Programmatic efforts to identify CKD biomarkers

Independent laboratories have labored since 2004 using proteomic approaches to identify intact proteins or peptide fragments that outperform serum creatinine as a predictor of renal function decline and CKD. Recently, Krochmal et al. performed a review of all publications that used various proteomic and peptidomic approaches to identify proteins and protein fragments that correlate with CKD progression [6]. Studied sample types include disparate body fluids, such as urine plasma and serum as well as kidney tissue. While the inclusion of data from disparate sources might appear to introduce confounders, it is essential to identify common peptides associated with CKD. Inclusion criteria were peptidomics or proteomics case–control studies relative to CKD and only studies that examined human specimens. In addition to the literature
search, the authors queried several databases including the ‘Chronic Kidney Disease database’ [7], ‘The Kidney and Urinary Pathway Knowledge Base’ [8], and the ‘Human Urinary Proteome Fingerprint Database’ to identify proteomic data that might produce CKD biomarker candidates [9–11]. The authors identified 114 manuscripts in their literature mining that fit inclusion criteria. Between the manuscripts and examining existing databases, 415 nonredundant peptide sequences from peptidomic studies and 4225 unique proteins from proteomics experiments were the most identified. The most commonly represented peptides (from peptidomic studies) were sequences of collagen alpha-1(I), collagen alpha-1(III), alpha-1-antitrypsin, and uromodulin. The common protein findings from proteomic experiments included Ig kappa chain V-IV, serum albumin, kininogen-1, and the protein AMBP. These findings have been entered in the PeptiCKDdb database [12]. This will provide a valuable tool to aggregate proteomic-identified candidate biomarkers of CKD.

Many of the peptides in the PeptiCKDdb database that correlate with CKD were identified by the group working at Mosaïques Diagnostics GmbH who used capillary electrophoresis coupled to mass spectrometry (CE-MS) to identify primarily urine peptides associated with progressive CKD. They have developed a testing system that uses a panel of 273 urine peptides (CKD273) that predicts the decline of glomerular filtration at a point early in the progression of CKD [13]. The CKD273 panel has been shown to predict the onset of microalbuminuria in patients with type 2 diabetes and when fully validated would allow clinicians to intervene at an early stage to prevent diabetic nephropathy [14,15]. The CKD273 biomarker appears to have predictive value of CKD progression in diabetics when estimated glomerular filtration rate is 70–80 mL/min and for those patients its predictive value exceeds urinary albumin excretion. If confirmed, this and other biomarkers with the ability to define those diabetic patients most at risk for CKD would be very useful in that it would allow clinicians to focus resources such as diabetes managers, nutritionists, and other clinicians on those patients most likely to have progressive CKD. Because the clinical assay requires analysis by CE-MS (as it did in the discovery phase), it will be challenging to make it available on a large scale; however, the inventors have demonstrated the reproducibility and precision of CE-MS of CKD273 in different platforms in disparate geographic locales [16].

3. Difficult lessons learned

The most notable programmatic effort to find CKD biomarkers and alternatives to creatinine is an NIH-sponsored consortium. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) issued a request for applications in 2009 for the Chronic Kidney Disease Biomarker Discovery and Validation Consortium (CKD BioCon). The request for applications proposed ‘Discovery of new biomarkers in body fluids (serum, urine, saliva, tears) or tissues from well-characterized patients with stable or progressive CKD and appropriate controls using genomic, proteomic or metabolomic techniques, to assess structure, function, injury, repair, and progression of chronic kidney damage.’ The CKD BioCon entered the field of biomarker discovery with considerable assets that included access to 58,000 specimens, multidisciplinary expertise in nephrology, proteomics, clinical chemistry, epidemiology, and informatics [17]. To date, several studies have been performed by the CKD BioCon both examining previously identified biomarker candidates and performing proteomic-based discovery. In general, the studies performed using previously identified biomarker candidates have not outperformed serum creatinine [18–20]. The proteomic-driven discovery studies performed by the consortium have focused on prediction of disease flares in autoimmune CKD [21,22]. Despite a surfeit of negative findings by the CKD BioCon consortium, a number of valuable lessons have been learned as to the structure of renal biomarker studies for proteomic analysis and development of new mass spectrometry-based assays. These include the proper collection, processing and storage of specimens from large cohorts for proteomic analysis, development of standard operating procedures for multiple reaction monitoring, external quality control for assays including blind duplicates, and proficiency samples from quality control pools [17]. Substantial progress has been made in developing multiple reaction monitoring assays in urine, particularly of the candidate CKD biomarker uromodulin [22,23]. While CKD BioCon has not produced any breakthrough CKD biomarkers to date, it clearly has set standards for programmatic efforts to identify renal disease biomarkers and raised standards to those used in successful cancer biomarker consortiums.

4. Emerging successes in CKD biomarker identification

In reviewing the discovery-oriented proteomic approaches, it is clear that they have not identified CKD biomarkers that have been validated and implemented clinically with the possible exception of CKD273, which has been issued a letter of support from the FDA to encourage further development [13]. However, a more targeted proteomic approach has yielded the only FDA-approved biomarker of a CKD. Beck et al. [24] used a mass spectrometry-based approach to identify the target antigen of autoantibodies in membranous nephropathy (MN). For many years, it had been known that the sera of patients with MN contained antibodies to protein(s) present in the glomerulus. Patient sera containing the autoantibodies were used as the primary antibody in immunoblots of normal renal glomeruli. The corresponding gel bands were excised and analyzed by mass spectrometry. Ultimately, the target antigen was identified as the M-type phospholipase A2 receptor (PLA2R). Since that work was first published in 2009, an assay for anti-PLA2R antibodies has gained the FDA approval as a test in the differential diagnosis of MN or nephrotic syndrome of unknown etiology. Approximately 80% of patients with MN express anti-PLA2R antibodies, and the titer of these antibodies may correlate with disease activity and the response to therapy [24]. Using a similar approach, Tomas et al. identified a second autoantigen active in approximately 10% of MN patients [25].

In line with more targeted approaches, the use of laser microdissection of renal tissue coupled to mass spectrometry
has begun to provide new insights and is a promising approach to define new CKD biomarkers [26,27].

5. The challenge and the promise of applying proteomics to CKD biomarker discovery

In reviewing this literature, it is possible to conclude that proteomic methods have produced very little magic in the field of CKD biomarkers. But, it is good to remember two things before accepting that judgment. First, the discovery of creatinine, the CKD biomarker gold standard, required 40 years from the optimization of an assay to the discovery that it could be used as a surrogate marker of kidney function. We are approximately 13 years into our attempts to discover CKD biomarkers. As a discipline, we have discovered one clinically implemented biomarker after just 6 years of effort. Second, we must have the insight and humility to acknowledge that proteomic methods inevitably performed subject to the dictates of the futurist Roy Amara, whose law of technology states:

‘We tend to overestimate the effect of a technology in the short run and underestimate the effect in the long run.’

Amara’s law is frequently expressed graphically as the ‘Hype Cycle of Emerging Technologies’ (Figure 1). I suspect that as we look back in a number of years that we will accept that we have passed the Peak of Inflated Expectations, are passing through the Trough of Disillusionment, and are entering the Slope of Enlightenment.

### Funding

The author was supported by The University of Louisville School of Medicine.

### Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

### References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (–) to readers.

1. Zerhouni E. Medicine. The NIH roadmap. Science. 2003;302 (5642):63–72.
2. Woodcock J, Woosley R. The FDA Critical Path Initiative and its influence on new drug development. Annu Rev Med. 2008;59:1–12.
3. American Society of Nephrology Renal Research Report. J Am Soc Nephrol. 2005;16(7):1886–1903.
4. Schaub JA, Parikh CR. Biomarkers of acute kidney injury and associations with short- and long-term outcomes. F1000Res. 2016;5:986.
5. Rehberg PB. Studies on kidney function. Rate Filtration Reabsorption Human Kidney. 1926;20(3):447–460.
6. Krochmal M, Fernandes M, Filip S, et al. PeptiCKDdb-peptide- and protein-centric database for the investigation of genesis and progression of chronic kidney disease. Database (Oxford). 2016.
7. Website: Chronic Kidney Disease database CKDdb. Publisher: Identification of Molecular Determinants of Established CKD (iMODE-CKD) Consortium. Available from: http://www.ckddb.org
8. Website: The Kidney and Urinary Pathway Knowledge Base. Publisher: e-Laboratory for Interdisciplinary Collaborative Research in Data Mining and Data-Intensive Science (e-LICO). Available from: http://www.kupkb.org
9. Fernandes M, Husi H. FP222The chronic kidney disease database (CKDdb). Nephrol Dial Transplant. 2015;30(suppl3):iii141–iii141.
10. Husi H, Barr JB, Skipworth RJ, et al. The human urinary proteome fingerprint database UPdb. Int J Proteomics. 2013;2013 760208:1–7.
11. Klein J, Jupp S, Moulos P, et al. The KUPKB: a novel web application to access multilomics data on kidney disease. Faseb J. 2012;26(5):2145–2153.
12. Website: Peptide and Protein-centric Database. Publisher: Identification of Molecular Determinants of Established CKD (iMODE-CKD) Consortium. Available from: http://www.peptickddb.com/
13. Nkuipou-Kenfack E, Zurbrig P, Mischak H. The long path towards implementation of clinical proteomics: exemplified based on CKD273. Proteomics Clin Appl. 2016.
14. Lindhardt M, Persson F, Zurbrig P, et al. Urinary proteomics predict onset of microalbuminuria in normoalbuminuric type 2 diabetic patients, a sub-study of the DIRECT-Protect 2 study. Nephrol Dial Transplant. 2016.
15. Pontillo C, Jacobs L, Staessen JA, et al. A urinary proteome-based classifier for the early detection of decline in glomerular filtration. Nephrol Dial Transplant. 2016.
16. Description of CKD273 biomarker.
17. Mischak H, Vlahou A, Ioannidis JP. Technical aspects and inter-laboratory variability in native peptide profiling: the CE-MS experience. Clin Biochem. 2013;46(6):432–443.
18. Hsu CY, Ballard S, Batlle D, et al. Cross-disciplinary biomarkers research: lessons learned by the CKD biomarkers consortium. Clin J Am Soc Nephrol. 2015;10(5):894–902.
19. Important conclusions derived from the organization and early experience in the CKD Biomarkers Consortium.
20. Rebolhoz CM, Grams ME, Coresh J, et al. Serum fibroblast growth factor-23 is associated with incident kidney disease. J Am Soc Nephrol. 2015;26(1):192–200.
21. Foster MC, Coresh J, Hsu CY, et al. Serum beta-trace protein and beta2-microglobulin as predictors of ESRD, mortality, and cardiovascular disease in adults with CKD in the Chronic Renal Insufficiency Cohort (CRIC) study. Am J Kidney Dis. 2016;68(1):68–76.
22. Fufaa GD, Weil EJ, Nelson RG, et al. Association of urinary KIM-1, L-FABP, NAG and NGAL with incident end-stage renal disease and mortality in American Indians with type 2 diabetes mellitus. Diabetologia. 2015;58(1):188–198.
23. Rovin BH, Klein JB. Proteomics and autoimmune kidney disease. Clin Immunol. 2015;161:23–30.
22. Abulaban KM, Song H, Zhang X, et al. Predicting decline of kidney function in lupus nephritis using urine biomarkers. Lupus. 2016;25(9):1012–1018.

23. Fu Q, Grote E, Zhu J, et al. An empirical approach to signature peptide choice for selected reaction monitoring: quantification of uromodulin in urine. Clin Chem. 2016;62(1):198–207.

24. Beck LH, Jr., Bonegio RGB, Lambeau G, et al. M-Type Phospholipase A2 Receptor as Target Antigen in Idiopathic Membranous Nephropathy. New England Journal of Medicine. 2009;361(1):11–21. *First description of only FDA-approved CKD biomarker discovered by proteomic approaches.*

25. Tomas NM, Beck LH Jr., Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371(24):2277–2287.

26. Hobeika L, Barati MT, Caster DJ, et al. Characterization of glomerular extracellular matrix by proteomic analysis of laser-captured microdissected glomeruli. Kidney Int. 2017;91(2):501–511.

27. Nasr SH, Dasari S, Hasadsri L, et al. Novel type of renal amyloidosis derived from apolipoprotein-CII. J Am Soc Nephrol. 2017;28(2):439–445.

28. Jeremy Kemp at English Wikipedia, CC BY-SA 3.0. [https://commons.wikimedia.org/w/index.php?curid=10547051](https://commons.wikimedia.org/w/index.php?curid=10547051)