Smad1 as a Biomarker for Diabetic Nephropathy

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Diabetic nephropathy is one of the most devastating complications of diabetes and develops in about one-third of diabetic patients. Exposure to a hyperglycemic environment with coexisting obesity, metabolic syndrome, hyperfiltration, hypertension, and hyperlipidemia plays a critical role in the development of diabetic nephropathy (1,2). Clinical trials demonstrated that improving glycemic control delays and, in some cases, may prevent the subsequent development of diabetic nephropathy. The concept that selected individuals with diabetes (at similar glycemic control) are at different risk for developing nephropathy was developed about 20 years ago after finding familial aggregation of kidney disease (3,4). Detailed phenotyping studies indicate that diabetic nephropathy progression and patients' responses to treatment also varies significantly between individuals.

To identify patients at increased risk for the development of diabetic nephropathy, considerable scientific effort has been dedicated to developing new screening and prognostic markers (5). The hope is that these new biomarkers will predict the development of the disease earlier than the currently used disease marker, albuminuria. The problem with albuminuria as a disease marker is twofold. Low-grade albuminuria (microalbuminuria) is a poor predictor of diabetic nephropathy (6); high-grade albuminuria, which is a strong predictor of disease progression, only develops at advanced diabetic nephropathy, a stage when less can be done to prevent the development of end-stage kidney failure.

How can we identify biomarkers? Traditionally, biomarker identification has mostly been a one-at-a-time approach. Many well-known tests have been identified based on clear biological insight from physiology or biochemistry. One example of this path was the discovery that inulin infusion measures glomerular filtration rate. Based on these studies, investigators discovered a naturally occurring molecule, creatinine, that enabled the same measurements. Biomarkers validated by genetic and molecular biology methods can be classified into different types. Screening or predicting biomarkers are used to identify people who are at risk for the development of the disease. Diagnostic markers are used to diagnose a certain condition. Prognostic markers can predict the outcome or the natural history of a disease, and drug activity markers can predict treatment response.

In recent years, biomarker discovery has been booming, mainly due to two independent factors. First, there has been a considerable shift in clinical medicine from trying to treat chronic debilitating conditions (including diabetes, cancer, and renal disease), which has not been very successful, to early diagnosis and prevention, which has been far more successful in the area of various cancer types and cardiovascular disease. The other factor that significantly contributed to this boom is the shift in the biomarker discovery pipeline from the one-at-a-time approach to a far more efficient high–through-put test approach (7–9). Thus genetics, genomics, proteomics, and metabolomics are preferentially used in biomarker discovery.

All of these methods are currently applied to discovering new biomarkers of diabetic nephropathy. Large genetics trials (including the Family Investigation of Nephropathy and Diabetes [FIND] and Genetics of Kidneys in Diabetes [CoKinD] studies) have been conducted to find genetic polymorphisms associated with diabetic nephropathy (10–12). These clinical trials identified multiple loci associated with increased risk of diabetic nephropathy, indicating that it is a polygenic disease with multiple genes each involved with possible small effects. It is expected that with the increased availability of whole genome scan arrays, new loci will be identified and candidate polymorphisms will soon be established. Many new high–through-put functional genomic methods have been recently developed and are currently being applied to identify new biomarkers for diabetic nephropathy. The advantage of functional genomics (mainly microarrays) is that methodologies are fairly well established and robust. In addition, as most microarray studies are performed in kidney biopsy tissue, the diagnostic gold standard (the histology) is available, and gene expression levels can be directly correlated with the renal morphology. It is also very likely that gene expression changes observed in the kidney will also play role in disease pathogenesis, thereby becoming a therapeutic target (13,14). However, kidney tissue is not readily available, as current clinical practice avoids kidney biopsies in diabetic patients. Therefore, although these tissue-based approaches clearly revolutionalized the cancer field, the greatest question for us is whether the marker sets will have high enough predictive value to justify the performance of a high-risk procedure (kidney biopsy) in diabetic patients at the stage of essentially normal renal function.

Proteomic and, lately, metabolomic analyses probably hold the greatest promise to identify a new diabetic nephropathy biomarker that can be rapidly translated to clinical medicine (15,16). This promise derives from the current practice that most available diagnostic tests are either blood- or urine-based and involve the detection of proteins, enzyme activity, or small molecule levels. Urine represents a modified ultrafiltrate of plasma, with protein concentrations about 1,000-fold lower than plasma with increased proportions of low–molecular-weight proteins, and it is also enriched in proteins released along the

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urinary tract. Different proteomic methods are currently being tried to identify a urinary biomarker of renal disease, but most methods do not offer the through-put currently obtained with genomic methods. In addition, many biologically interesting molecules are low-abundance proteins, whereas the 22 most abundant proteins constitute 99% of the total protein mass (in the blood and the urine). Despite all the challenges, few published studies using urinary proteomic analysis indicate the feasibility of the approach (17). Metabolomics is also receiving increasing attention as recent estimates suggest that the human metabolome comprises ~3,000 small molecules, which is many orders of magnitude lower than the number of transcripts and proteins (10^5).

While these large-scale nonbiased approaches were being developed and conducted, Mima et al. (18) performed a hypothesis-driven study using well-established mouse and rat models of diabetic nephropathy. Previously, they showed that Smad1 plays an important role in the development of mesangial expansion. Now they invesitgate whether urinary Smad1 levels measured at the time of diabetes development correlate with the development of mesangial expansion at later time points. To make their point, they used not only type 1 diabetic rats but also type 2 diabetic *db/db* mice. In addition, to mimic the human situation, some animals were treated with the angiotensin receptor inhibitor olmesartan, which is known to block diabetic nephropathy development. They show a very good correlation between urinary Smad1 levels and the development of mesangial expansion ($r = 0.7$ and 0.62 in rats and mice, respectively), whereas the correlation between albuminuria and mesangial expansion was not statistically significant. Interestingly, blood glucose and blood pressure levels observed before the development of diabetes did not correlate with mesangial expansion development. These observations are intriguing and (if they can be translated to humans) could represent a real breakthrough. Of course, there are a number of critical issues here, as it is well known that rodent models of diabetic nephropathy do not faithfully replicate the human condition because they do not show decline in renal function, which is the most critical parameter for measuring progression in humans.

The glass of diabetic nephropathy biomarker research is finally half full, and significant challenges are ahead of us. Based on the complex disease pathogenesis, it is likely that a group of markers will be needed to predict end-stage renal disease development. Additional complicating factors are that disease development and disease activity/progression and treatment response might be best predicted by different marker sets. Despite the promise and excitement of the high–through-put methods, we also need to keep in mind that small-scale hypothesis/pathomechanism driven methods have been the basis for our biomarker discovery for decades. While it is a long journey to translate findings of small-scale studies to clinical practice, we are now realistically looking forward to new findings and developments as the diabetic nephropathy biomarker field unfolds.

REFERENCES

1. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N Engl J Med* 342:381–389, 2000
2. Colwell JA: Intensive insulin therapy in type II diabetes: rationale and collaborative clinical trial results. *Diabetes* 45 (Suppl 3):S87–S90, 1996
3. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320:1161–1165, 1989
4. Jones CA, Krolewski AS, Rogus J, Xue JL, Collins A, Warram JH: Epidemic of end-stage renal disease in people with diabetes in the United States population: do we know the cause? *Kidney Int* 67:1684–1691, 2005
5. Susztak K, Bottinger EP: Diabetic nephropathy: a frontier for personalized medicine. *J Am Soc Nephrol* 17:361–367, 2006
6. Perkins BA, Ficociello LH, Ostrander BE, Silva KH, Weinberg J, Warram JH, Krolewski AS: Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. *J Am Soc Nephrol* 18:1353–1361, 2007
7. Lockhart DJ, Winzeler EA: Genomics, gene expression and DNA arrays. *Nature* 405:827–836, 2000
8. Iyengar SK, Schelling JR, Sedor JR: Approaches to understanding susceptibility to nephropathy: from genetics to genomics. *Kidney Int* 61:61–67, 2002
9. Gershon D: Microarray technology: an array of opportunities. *Nature* 416:885–891, 2002
10. Mueller PW, Rogus JJ, Cleary PA, Zhao Y, Smiles AM, Steffes MW, Bucksa J, Gibson TB, Cordovado SK, Krolewski AS, Nierras CR, Warram JH: Genetics of Kidneys in Diabetes (GoKinD) study: a genetics collection available for identifying genetic susceptibility factors for diabetic nephropathy in type 1 diabetes. *J Am Soc Nephrol* 17:1782–1790, 2006
11. Iyengar SK, Abboud HE, Goddard KA, Saad MF, Adler SG, Arar NH, Bowden DW, Duggirala R, Elston RC, Hanson RL, Ipp E, Kao WH, Kimmel PL, Klag MJ, Knowler WC, Meoni LA, Nelson RG, Nicholas SB, Pahl MV, Parekh RS, Quade SR, Rich SS, Rotter JI, Scavini M, Schelling JR, Sedor JR, Sehgal AR, Shah VO, Smith MW, Taylor KD, Winkler CA, Zager PG, Freedman BI, Family Investigation of Nephropathy and Diabetes Research Group: Genome-wide scans for diabetic nephropathy and albuminuria in multiethnic populations: the Family Investigation of Nephropathy and Diabetes (FIND). *Diabetes* 56:1577–1585, 2007
12. Schelling JR, Abboud HE, Nicholas SB, Pahl MV, Sedor JR, Adler SG, Arar NH, Bowden DW, Elston RC, Freedman BI, Goddard KA, Guo X, Hanson RL, Ipp E, Iyengar SK, Jun G, Kao WH, Kasinath BS, Kimmel PL, Klag MJ, Knowler WC, Nelson RG, Parekh RS, Quade SR, Rich SS, Saad MF, Scavini M, Smith MW, Taylor K, Winkler CA, Zager PG, Shah VO, Family Investigation of Nephropathy and Diabetes Research Group: Genome-wide scan for estimated glomerular filtration rate in multi-ethnic diabetic populations: the Family Investigation of Nephropathy and Diabetes (FIND). *Diabetes* 57:235–243, 2008
13. Kretzler M, Cohen CD, Doran P, Henger A, Madden S, Grone EF, Nelson PJ, Schlondorff D, Grone HJ: Repuncturing the renal biopsy: strategies for molecular diagnosis in nephrology. *J Am Soc Nephrol* 13:1961–1972, 2002
14. Schmid H, Boucherot A, Yasuda Y, Henger A, Brunner B, Eichinger F, Nitsche A, Kiss E, Bleich M, Grone HJ, Nelson PJ, Schlondorff D, Cohen CD, Kretzler M: Modular activation of nuclear factor-κB transcriptional programs in human diabetic nephropathy. *Diabetes* 55:2993–3003, 2006
15. Thongboonkerd V, Barati MT, McLeish KR, Pierce WM, Epstein PN, Klein JB: Proteomics and diabetic nephropathy. *Contrib Nephrol* 141:142–154, 2004
16. Sharma K, Lee S, Han S, Lee S, Francos B, McCue P, Wassell R, Shaw MA, RamachandraRao SP: Two-dimensional fluorescence difference gel electrophoresis analysis of the urine proteome in human diabetic nephropathy. *Proteomics* 5:2648–2655, 2005
17. Mischak H, Kaiser T, Walden M, Hillmann M, Wittke S, Herrmann A, Knueppel S, Haller H, Fliser D: Proteomic analysis for the assessment of diabetic renal damage in humans. *Clin Sci (Lond)* 107:485–495, 2004
18. Mima AAH, Matshubara T, Abe H, Nagai K, Tamura Y, Torikoshi K, Araki M, Kanamori H, Takahashi T, Tominaga T, Matsuura M, Iehara N, Fukatsu A, Kita T, Doi T: Urinary Smad1 is a novel marker to predict later onset of mesangial matrix expansion in diabetic nephropathy. *Diabetes* 57:1712–1722, 2008