Aptamer-Based Proteomic Platform for Human Immune-Mediated Kidney Diseases

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Standard diagnostic methods for human immune-mediated kidney diseases are undergoing a major transition. Membranous nephropathy (MN) is the most frequent cause of primary nephrotic syndrome among adults. PLA2R, a transmembrane glycoprotein expressed in human podocytes, and thrombospondin type 1 domain-containing 7A, another podocyte-expressing protein, were identified as a target antigen of idiopathic MN in 2009[1] and 2014,[2] respectively. Of note, autoantibody result against PLA2R is positive in the blood of 60% to 70% of patients with MN. According to the Kidney Disease: Improving Global Outcomes Clinical Practice Guidelines, kidney biopsy is no longer required to diagnose MN in patients presenting nephrotic syndrome and a positive anti-PLA2R antibody result.[3] Moreover, monitoring anti-PLA2R antibody titers might be useful for evaluating treatment response and adjusting treatments.[3] Unfortunately, this approach can be applied to a limited number of adult patients presenting nephrosis, and therefore most of those patients still require renal biopsy for diagnosis. Therefore, researchers have attempted to identify novel blood or urine biomarkers for several years, given their convenience and low invasiveness. In contrast, the diagnosis of minimal change disease (MCD), a common cause of nephrotic syndrome among all generations, remains dependent on its clinical characteristics, histopathologic findings of the kidney specimens, and response to corticosteroid therapy, although renal biopsy might not be essential in pediatric patients. However, the specific serologic characteristics of MCD remain undefined.

For most human immune-mediated kidney diseases except disorders described previously, histopathologic evaluation with a needle kidney biopsy remains the blueprint for a definitive diagnosis. However, in patients with uncontrollable bleeding tendency, uncooperative behavior, or severe obesity, a biopsy cannot be indicated in clinical practice owing to the invasive nature of the procedure. To overcome this issue, a variety of omics approaches have been implemented to identify unknown biomarkers for immune-mediated kidney diseases in an unbiased manner. Therein, protein is an attractive target, as the proteome presents the terminal output of genome, transcription, and epigenetic modifications, and protein-targeted research is expected to generate precise information regarding diseases at a specific time point. Indeed, identification of the former PLA2R remains the most clinically relevant and successful application of proteomics in nephrology to date. Use of proteomics approaches, such as laser microdissection followed by tandem mass spectrometry, has also led to the discovery of additional proteins as target antigens of MN, including exostosin 1/exostosin 2, neural epidermal growth factor-like 1, semaphorin-3B, and protocadherin 7.[4]

In this issue, Muruve et al.[5] used quantitative SOMAscan and aptamer-based proteomics to identify serum protein biomarkers of MCD and MN (Figure 1). By analyzing serum samples from patients with biopsy-confirmed MCD and MN and healthy control participants using SOMAscan (SomaLogic, Boulder, CO), the authors identified 208 and 244 proteins that differentiated MCD and MN from healthy controls, respectively, among the 1305 proteins detected. Furthermore, 157 proteins could discriminate between these 2 glomerulopathies. In addition, hierarchical clustering and the second unsupervised learning method spotlighted a set of proteins that allowed excellent discrimination of patients with MCD from those with MN and healthy controls and could discriminate patients with MN from those with MCD or healthy controls.[5]

Aptamers are short, 20 to 80 nucleotides, single-stranded DNA or RNA sequences (nucleic acid

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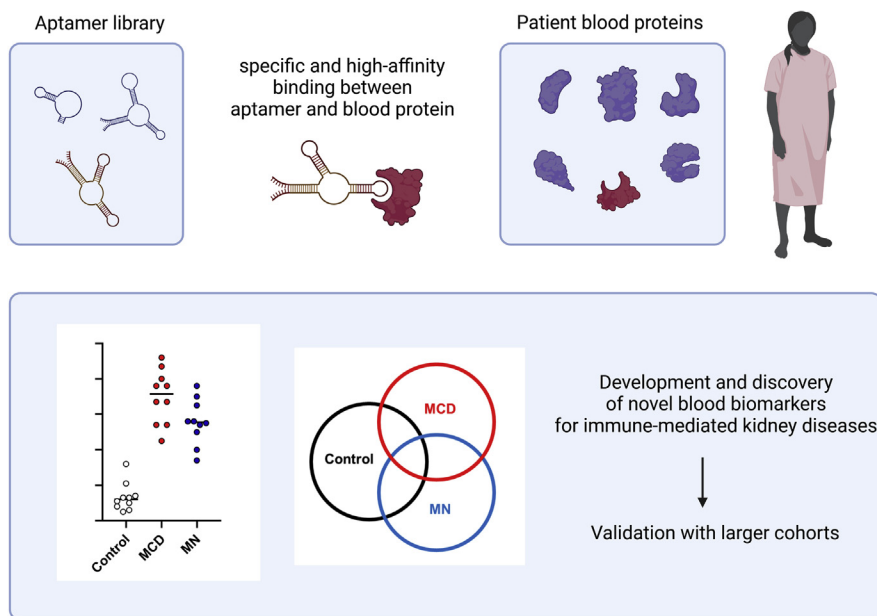
Figure 1. Strategy diagram of aptamer-based platform for blood protein biomarker discovery of human immune-mediated kidney diseases. Because a nucleic acid aptamer binds to a specific protein with high affinity, their quantitative result recapitulates the protein concentration. The aptamer library covers >1000 protein-capture modified DNA aptamers to bind to preselected clinically relevant proteins. When these engineered aptamers are applied to the blood samples obtained from healthy control participants and patients with immune-mediated kidney diseases, such as MCD and MN, the scanning reveals each protein abundance in blood samples of 3 different groups. By comparing those, the candidate for kidney disease biomarker proteins can be identified. The diagnostic value of candidate proteins should be assessed in larger validation cohorts. Created with BioRender.com. MCD, minimal change disease; MN, membranous nephropathy.

aptamers) or proteins (peptide aptamers) that bind to target molecules, such as proteins, cells, metal ions, and even small organic molecules, with high affinity and specificity. For example, in HIV, a short RNA ligand, called the transactivation response element, promotes transactivation and virus replication by binding with the viral Tat protein. The emergence of aptamers in the 1980s afforded an attractive alternative to antibodies. In 1990, the systematic evolution of ligands by exponential enrichment was established as a basic technique for isolating aptamers.[6] Nucleic acid aptamers that bind specifically to target molecules were generated using a nucleic acid library of approximately 1014 random sequences. After mixing the library nucleic acids and target molecules, nucleic acids bound to target molecules were amplified

using the polymerase chain reaction method for DNA or the reverse-transcriptase polymerase chain reaction method for RNA; then, sequences were determined by sequencing analysis and aptamers were identified by molecular interaction analysis. Aptamers are expected to be novel sensor elements that surpass antibodies, as they can be cost-effectively produced by chemical synthesis and used to detect small molecules that are difficult to obtain with antibodies. In addition, aptamers are being extensively studied from basic research to translational applications. SomaLogic Operating Co., Inc., modified the systematic evolution of ligands by exponential enrichment method from a chemical viewpoint to develop >1000 unique and artificial deoxynucleotide aptamers, named SOMAmers,

to bind specifically to human blood proteins with biomedical significance in their native conformation. Subsequently, SOMAscan can slow these off-rate modified aptamers and enable the analysis of approximately 7000 proteins from a small blood specimen. Given several biomarkers that serve as indicators reflecting disease activity and progression are blood proteins, tests using aptamer technology to accurately measure blood proteins, which are altered from time to time depending on the state of the body, are attracting attention. This increased capability is the major advantage of SOMAscan over conventional measures, in addition to its high sensitivity (median lower limit of detention of 40 fM), high reproducibility (median coefficient of variance <5%), and dynamic ranges.[5]

However, there exists scope for improvement considering the specificity of this approach.[7,8] It should be noted that the binding affinity of aptamers to proteins may be influenced by numerous molecular properties, such as protein structure, posttranslational modifications, genetic polymorphisms, and complex formation. For example, the specificity of aptamers of distinct proteins in the context of chronic kidney disease was assessed by comparing the results of SOMAscan with those from immunoassays.[8] Although some proteins had strong correlations with the observed results, others had inconsistencies. This indicates that SOMAscan is a powerful tool for initial protein screening; however, results need to be confirmed using different platforms, such as traditional antibody-based techniques, to complement its insufficient accuracy.

To mechanistically extend the results obtained using SOMAscan, Muruve *et al.*[5] further explored the underlying signaling pathways

in MCD and MN. The highly discriminatory proteins in the MCD signature had a prominent increase in immune and growth factor signaling proteins and in carbohydrate and lipid metabolism. Proteins in the MN signature are involved in inflammation, cytokines, and growth factor signaling. These alterations were also observed after additional pathway analysis. Furthermore, 49 MN-specific proteins that discriminate MN from MCD and healthy controls or 70 MCD-specific proteins that discriminate MCD from MN and healthy controls were set as inputs in the ingenuity pathway analysis. On the basis of pathway analysis, cell survival pathways were increased in MN and inflammatory proteins were predicted to be upstream regulators of MN-specific proteins. For MCD, cell death was decreased, whereas vascular function was increased, contrary to that observed in MN. Additional characteristics of MCD include decreased movement and immune cell activation.

Ingenuity pathway analysis is a software application used to analyze data derived from omics research, including proteomics. Ingenuity pathway analysis can help unveil the significance of data and identify regulators and pathways in the context of biological systems. The use of SOMAscan followed by ingenuity pathway analysis has previously been applied in nephrology research. One study has compared native and post-transplant patients with chronic kidney disease with healthy controls.[9] The authors revealed that angiogenesis-mediated and inflammation-mediated pathways are activated in patients with chronic kidney disease.[9] Another study focused on patients with dialysis-dependent acute kidney injury and compared the serum samples of patients who discontinued dialysis with those of

patients who remained on dialysis. Reportedly, alternations in activated or suppressed pathways, such as cellular growth, proliferation, and cell death, could potentially predict the kidney function recovery recovery.

Although dyslipidemia is a well-known manifestation of nephrotic syndrome, it is intriguing that dysregulation of fatty acid metabolism pathways has been documented in both MN and MCD.[5] Both immune-mediated kidney diseases may be associated with the facilitation of liver lipid metabolism secondary to hypoalbuminemia and injury to the kidney tubular cells. In addition, their pathway analysis nicely provides a novel hypothesis regarding the underlying pathophysiology; although, it is yet to be applied as a precise target in clinical settings. In addition to the requirement for confirming reproducibility and validation, pathway analysis fails to distinguish between primary pathogenesis and subsequent reactions, as highlighted by Muruve et al.[5] Furthermore, the analysis of blood samples does not provide direct information regarding the specific site of pathway dysregulation.

In sum, Muruve et al.[5] focused on a particular proteome in serum samples from patients with 2 different human immune-mediated kidney diseases. The authors identified a set of proteins specific to these kidney disorders and smartly revealed that cell death, inflammation, and fatty acid metabolism could be characterized by additional pathway analysis. Given the lack of specific and sensitive noninvasive biomarkers for the differential diagnosis of MCD or PLA2R-negative MN, their actual human sample-based approach is straightforward and powerful. Future work with a validation cohort will be required to further filter the biomakers.

DISCLOSURE

The author declared no competing interests.

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