Molecular nephropathology: ready for prime time?

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Adam B, Mengel M. Molecular nephropathology: ready for prime time?, Am J Physiol Renal Physiol 309: F185–F188, 2015. First published May 27, 2015; doi:10.1152/ajprenal.00153.2015.—In the current era of precision medicine, the existing nephropathology paradigm of light microscopy, immunofluorescence, and electron microscopy will become increasingly insufficient. There will be an expectation to supplement these traditional diagnostic tools with patient-specific information related to a growing understanding of molecular pathophysiology. Next generation sequencing technologies are expected to play a key role in the future of nephropathology, but transcriptomics is poised to represent the first major foray into routine molecular testing. The introduction of molecular techniques into clinical nephropathology has been hindered in part by the reliance of existing platforms on fresh tissue samples. The NanoString gene expression system works with formalin-fixed paraffin-embedded tissue and thus represents a promising solution to this technical barrier that may finally allow for the translation of recent transcriptomics discoveries into the enhancement of patient care. Widespread adoption of this new diagnostic dimension will require ongoing multidisciplinary cooperation between pathologists and clinicians, including molecular testing consensus generation and rigorous multcenter validation.

gene expression; molecular; NanoString; nCounter; nephropathology

Challenges and Opportunities in Contemporary Nephropathology

Many renal diseases continue to be classified by histological, immunological, and clinical correlates that do not necessarily represent underlying pathophysiological mechanisms (8). Despite its strengths, the evaluation of histomorphology is limited by the subjective interpretation of lesions with nonspecific etiological association (5). Quality assurance studies have repeatedly demonstrated suboptimal inter- and intraobserver reproducibility in the assessment of renal biopsies (1, 13, 14, 30, 33). These limitations in diagnostic precision ultimately compromise our ability to effectively treat patients and accurately define research endpoints (35).

The traditional nephropathology toolbox of light microscopy, immunofluorescence, and electron microscopy is becoming increasingly insufficient in the modern era of precision medicine. Completion of the Human Genome Project in 2003 and subsequent development of high-throughput molecular testing techniques have opened the door to enhanced diagnostic accuracy and personalized therapeutics (32, 36). These technological advancements have facilitated the assessment of molecular targets, including DNA, RNA, proteins, and metabolites, and are expected to accelerate the discovery and adoption of novel biomarkers for routine clinical application.

Illustrations of this generational shift can be seen in all areas of medicine, including nephropathology. In the 2013 Banff Classification of Renal Allograft Pathology, endothelial injury gene expression was added as a diagnostic criterion for antibody-mediated rejection (ABMR) (16). This is reflective of anticipated future directions in the field and has resulted in an urgent need for molecular diagnostics to be translated from ‘bench-to-bedside’ into routine clinical nephropathology.

Foundations of a New Diagnostic Dimension

A compelling example of increased molecular knowledge permitting improved diagnostics, prognostics, and theranostics in the field of nephropathology is the recently revised classification of membranoproliferative glomerulonephritis (MPGN). This group of diseases was traditionally separated into types I, II, and III depending on the location and characteristics of ultrastructural deposits. It is now known that a subset of types I and III with exclusive C3 deposits are etiologically mediated by dysregulation of the alternative complement pathway (48). These cases are now referred to as C3 glomerulonephritis, which, along with type II (dense deposit disease), fall within the larger category of C3 glomerulopathy. Various genetic mutations have been described in these patients, with the identification of specific aberrations enabling the administration of personalized targeted treatments (i.e., complement factor H-based therapy in the presence of related gene mutations) (40).

Significant progress has also been made in the understanding of focal segmental glomerulosclerosis (FSGS). Despite common morphological patterns, FSGS is now known to represent a heterogeneous group of podocytopathies associated with numerous genetic aberrations (44). Classical high-penetration mutations involving nephrin, podocin, and WT1 were initially identified with the use of early molecular techniques requiring significant effort and expense. However, the subsequent development of increasingly powerful high-throughput next generation sequencing (NGS) methods has allowed for the identification of additional complex mutations within the FSGS spectrum. Greater understanding of the molecular pathology in these patients will allow for potential targets of therapeutic intervention to be elucidated (22).

NGS technologies can be expected to provide similar novel insights for other genetic entities with overlapping histological phenotypes, such as Alport syndrome and the hereditary cystic kidney diseases. Complex mutations in large genes like those involving COL4A3-5 in Alport syndrome can be more efficiently deciphered with modern methodologies. This will allow for a more improved clinical stratification than is possible with histomorphology alone (28, 53).

Unfortunately, the genetic basis of many renal diseases remains largely unknown, making these powerful sequencing-based methodologies unlikely to be of widespread clinical utility in the near future. Until this knowledge gap is bridged,
measurement of the relative expression of particular constitutive genes will likely prove to be the most instructive. Transcriptomics, or gene expression quantification, is therefore expected to have the largest immediate impact in clinical nephropathology, which is reflected by the adoption of this methodology in the most recent Banff classification (16).

Transcriptome-wide microarray data from various research groups has defined the molecular phenotype of many human renal diseases (2, 17, 18, 24, 45, 46, 52). Sets of transcripts have been associated with specific histopathological patterns and diagnoses. Many of these studies have been performed on renal allograft biopsies, resulting in specific gene signatures being described for the prediction of donor organ performance (11, 19, 25–27, 37) as well as the diagnosis of ABMR (9, 20, 21, 47, 50), T cell-mediated rejection (41, 42), polyomavirus nephropathy (29), and chronic allograft damage (6, 12, 34). Although comparatively less has been done in the area of native renal disease, studies assessing acute kidney injury and primary glomerulonephritis have elucidated transcript sets that warrant further investigation as potential ancillary diagnostic tools (4, 23, 51). Despite these tremendous transcriptomics discoveries, however, translation into routine clinical practice has not yet been realized.

Molecular Nephropathology: Ready for Prime Time?

A significant barrier to the implementation of molecular diagnostics in clinical nephropathology has been the dependence of traditional platforms, like microarrays and quantitative reverse transcription polymerase chain reaction (qRT-PCR), on dedicated stabilized tissue samples. These must typically be procured in addition to the formalin-fixed, paraffin-embedded (FFPE) biopsies used for standard-of-care histological assessment, resulting in increased complexity, expense, and risk to patients (3, 31).

A novel high-throughput gene expression platform has recently become available that is reported to work reliably with archival FFPE tissue (Fig. 1) (38, 43, 54). The NanoString nCounter Analysis System (NanoString Technologies, Seattle, WA) utilizes a barcode-labeled probe-based methodology that, through a unique digital imaging counting step, provides direct quantification of molecular targets in a particular sample (15). The short 100-base pair probe sequences are appropriate for the fragmented nucleic acid targets typical of FFPE tissue, while maintaining adequate specificity. The technology allows for highly multiplexed analysis, with the ability of evaluating up to 800 customizable targets per sample. It has been shown to be highly reproducible across a broad dynamic range and to have superior sensitivity to microarrays and comparable sensitivity to quantitative PCR (15, 43). The assay is technically simpler than other gene expression platforms, with as little as 15 min of hands-on time and a total assay turnaround time of less than 24 h. Data normalization procedures have also been made comparatively more user-friendly with the availability of platformspecific analysis software. Depending on the number of targets analyzed, sample cost has the potential to be a fraction of other platforms, particularly microarrays, making it more feasible for clinical implementation.

Although FFPE-derived nucleic acid deterioration has been well described (56), the NanoString system has been optimized and validated for such material (38, 43). One reason for this advancement over existing gene expression platforms is the elimination of a reverse transcription step, which is known to introduce variability and bias (7). Despite the absence of amplification, relatively small amounts of nucleic acid material (~100 ng) are required. This means that only a few additional sections need to be obtained from existing FFPE renal biopsy blocks, with no impact on what is currently available for histological assessment. Being able to perform molecular testing on the same sample evaluated under the microscope also allows for direct histological-molecular correlation. Many of the described nephropathology-related transcript sets are specific to particular cell types that may, for example, be limited to the cortical structures of renal biopsies. The ability to confirm the appropriateness of the tissue submitted for molecular testing is a significant benefit over platforms requiring additional, dedicated, non-FFPE samples.

Current commercially available assays for the NanoString platform include DNA, mRNA, and microRNA. Use of this system for the analysis of protein targets has also recently been described (55) and is expected to eventually be made available to consumers. As an illustration of its potential for clinical implementation, the NanoString system has been validated and FDA approved for use as a gene expression-based risk strati-
fication tool in the treatment planning of breast cancer patients (10, 38). The practical implications of this technology for the field of nephropathology include access to an expanded scope of research through retrospective molecular analysis of a massive worldwide archive of well-annotated and clinically followed FFPE renal biopsies. From a clinical perspective, it offers the ability to integrate molecular testing into existing FFPE-based pathology workflows through the procurement of only a few additional sections. A compelling example of this potential utility is the use of microRNA expression profiling to help distinguish acute pyelonephritis from renal allograft rejection (39). This technology can also be utilized for less invasive approaches to the molecular assessment of renal disease through analysis of urine or peripheral blood (49).

Conclusion

In the current era of precision medicine, the existing nephropathology paradigm of light microscopy, immunofluorescence, and electron microscopy will become increasingly insufficient. There will be an expectation to supplement these traditional diagnostic tools with patient-specific information related to a growing understanding of molecular pathophysiology. NGS technologies are expected to play a key role in the future of nephropathology, but transcriptomics is poised to represent the first major foray into routine molecular testing. The introduction of molecular techniques into clinical nephropathology has been hindered in part by the reliance of existing platforms on non-FFPE tissue. The NanoString gene expression system represents a promising solution to this technical barrier that may finally allow for the translation of recent transcriptomics discoveries into the enhancement of patient care. Widespread adoption of this new diagnostic dimension will require ongoing multidisciplinary cooperation between pathologists and clinicians, including molecular testing consensus generation and rigorous multicenter validation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

B.A. prepared figure; B.A. and M.M. drafted manuscript; B.A. and M.M. edited and revised manuscript; B.A. and M.M. approved final version of manuscript.

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