From Laboratory to Clinic

Molecular diagnostics encompasses the development of new, molecular-based detection methods for infectious diseases, malignancies, and inherited disorders. Not long ago, diagnostic pathology was based on visual examination of tissue sections. While morphologic observation remains in wide use, aided by histochemical staining and other techniques, the incorporation of molecular biologic technologies is contributing to an understanding of disease processes at the molecular level. Molecular diagnostic testing services are in place in many hospital and reference laboratories and are increasingly being used to monitor treatment of infectious diseases and cancers, to determine disease susceptibility, and to characterize the pathogenicity and metastatic potential of cancers, and are expected to play an increasingly important role in drug development.

Technology Evolution

Andrea Ferreira-Gonzalez is Laboratory Director, Division of Molecular Diagnostics, in the Department of Pathology at Virginia Commonwealth University Health Systems, Richmond, VA. Her laboratory evaluates infectious diseases, inherited disorders, oncogene

copy number, and other gene abnormalities using PCR, DNA hybridization, restriction fragment-length polymorphism (RFLP), and blotting techniques, receptor assays, and other technologies. Having worked in the field for 17 years, she has observed an explosion in the last 11 driven by advances in the technology and acceptance of its clinical utility. Important advances include increasing specificity of tests and shorter turnaround times. In this university hospital setting, the menu of tests includes both commercial systems and in-house reagents. While there is not yet a complete system allowing fully automated processing from sample to results, they are moving some in-house developed assays to modified automated platforms and providing opportunities for involving university research laboratories in test development. She feels the maturation of and advances in molecular technologies, increased acceptance of clinical utility, lower costs, and increasing automation will allow molecular diagnostics to move into more diagnostic areas and diverse settings.

"The most demand in is infectious disease, where there are more data, and the clinical utility has major acceptance,

especially in virology, where organisms are hard to grow, for example, HIV, or impossible to culture, like hepatitis C (virus)," Gonzalez observes. They not only diagnose but also monitor patients during anti-viral therapy. Most molecular diagnostics in virology have a rapid turnaround using real-time technology. In oncology, diagnosis and monitoring are less advanced. Gonzalez says that, for infectious disease, most laboratories use commercial tests. In oncology, most laboratories develop their own tests. She observes that for some genetic tests developed in individual laboratories, there is no standardization. "It's a mixed bag. Some areas are standardized, some are not."

"Molecular diagnostics? My favorite topic," says Margaret L. Gulley, Associate Professor, Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, School of Medicine. Clinical laboratories in late 1980s started doing molecular diagnostics, first in the area of hematology, looking at lymphocyte clonality. Southern blots, the first molecular technique to be brought into the laboratory, is hardly used today, having been replaced by amplification and in situ techniques. Real-time amplification

Courtesy of Sandra M. Gaston, Director, Andrology Laboratory and Research Scientist, Beth Israel Deaconess Medical Center, Boston, MA.

Tissue prints generate a 2-dimensional map of cells from the surface of the specimen that can be used for protein and messenger RNA (mRNA) marker profiling and for DNA mutation analysis. (A) Tissue print from surface of slice of prostate specimen, resulting in imprint of cross-section of the gland at the level of the apex. Transfer of thin layer of material from fresh tissue to nitrocellulose can be monitored by direct visual inspection (traces of pathology marking inks applied to outside of specimen are visible). Area of print corresponding to peripheral zone of prostate stained with toluidine blue to visualize the microscopic layer of cells transferred from the specimen. (B) Distribution of stained cell nuclei shows both sheets of cells and tissue fragments bound to the nitrocellulose across the print field.

is now the hot topic as the technology evolves. Gulley predicts microarrays will be next. She notes that in the university hospital setting, besides hematologic pathology, the highest volume tests are in infectious disease and fluorescence in situ hybridization (FISH) for cancer and inherited disorders, followed by tissue typing. In the country as a whole, there is a high volume of identity testing to resolve child support issues and to solve crimes.

PCR is now a common platform, although it was slow to enter the clinical laboratory setting because of the risk of amplicon contamination. "Now we recognize how to control that, and the technology is more accepted in diagnostic laboratories," says Gulley. In the last 5 years, automation and the increasing use of kits have allowed some standardization of protocols and made it easier for smaller laboratories to begin to adopt molecular diagnostics. However, there remains a need for more standardization and better kits.

Gulley notes a lack of research funding, particularly on the part of manufacturers, to compare methods and determine the best markers, and anticipates government-funded projects will accomplish these tasks.

The Federal Initiative

The Division of Cancer Prevention Early Detection Research Network (EDRN) at the National Cancer Institute (NCI) was established with objectives including development and testing of promising biomarkers or technologies and collaboration among academic and industrial leaders in molecular biology, molecular genetics, clinical oncology, and clinical application for early cancer detection. The original 5-year awards were made to EDRN Biomarker Development Laboratories in 1999, with a second round in October 2004. "This initiative is new in the time scale of the NCI Division of Cancer Prevention," observes Martin G. Sanda, Director, Prostate Cancer Care Center, Beth Israel Deaconess Medical

Center, Boston, MA. "Cancer biomarkers will live or die depending on how they sort out in this NCI extramural program. In the coming years, the valuable biomarkers will be the ones that manage to percolate through this early validation network as markers from individual laboratories are methodologically sorted out into those that are ready for phase III clinical trials. With cancer biomarkers, the problem comes with validation. When applied more broadly, most sink," he comments.

Sanda's area of interest is prostate cancer, where one of the critical questions is how to distinguish aggressive cancers that warrant treatment from indolent cancers that lack near or intermediate term risk. Standard histopathology only goes so far to distinguish aggressive disease. One problem with using new molecular markers, Sanda points out, is that the amount of tissue obtained at biopsy is small, immunohistochemistry sometimes doesn't work well, and for some new markers there are no good antibodies.

Making a Mark

Sandra M. Gaston, Director, Andrology Laboratory and Research Scientist, Beth Israel Deaconess Medical Center, Boston, MA, and colleagues are developing technologies to overcome some of the problems associated with biomarker detection in biopsies. "You can't disrupt clinical care while looking at markers. Surgical pathologists get first dibs on the specimen," she says. "There are times when you can't get tissue, especially from tiny biopsies, even when you would most like to identify a marker." Her laboratory has developed tissue print and print-phoresis technologies to conserve tissue for clinical diagnosis without interrupting clinical care. Tissue printing involves transferring cells and extracellular matrix components from the tissue surface onto nitrocellulose membranes, generating a 2-dimensional anatomic image on which protein, RNA, and/or DNA molecular markers can be visualized using immunoblotting, nucleic acid hybridization, and other techniques.

Courtesy of Sandra M. Gaston, Director, Andrology Laboratory and Research Scientist, Beth Israel Deaconess Medical Center, Boston, MA.

Flow chart of spatial molecular profiling of tumor markers using tissue print and print-phoresis technologies. Duplicate tissue print collection (step 1) allows analysis of both messenger RNA (mRNA) and protein tumor markers from the same specimen while preserving the tissue for histopathological study. The distribution of mRNA markers can be determined by dividing the print into segments or "tiles" from which RNA is extracted for RT-PCR or microarray analysis (step 2). Focal concentrations of specific protein markers may be visualized directly on the primary prints; more complex molecular expression patterns are obtained from print-phoresis gels and Western blots (step 3). Histopathology (steps 4 and 5) may include both standard staining of the tissue sections and immunohistochemical studies. Marker maps derived from analysis of the tissue prints are then correlated with the histopathological features of specimen (step 6). L, left; R, right.

Using a nitrocellulose membrane applied to and peeled off the surface of a cancerous prostate after surgical removal, one can look for areas broken through the fibromuscular capsule layer, a sign of more aggressive disease. "The tissue print is like a big dot blot," Gaston explains. "But since you can't get the molecular weight from a dot blot, and there's also background, we cut the blot into strips, perform print-phoresis using polyacrylamide gel electrophoresis (PAGE) and get 75% to 80% recovery of markers." In looking for markers characteristic of invasive prostate cancers, they run zymography PAGE to generate collagen degradation product profiles, which are an indication of breakdown at the site of invasion of the capsule by the tumor. These techniques are also applicable to tiny needle biopsy specimens without damaging the tissue, which can still then be

examined by conventional techniques. "These micropeels have a tiny layer of cells wonderfully representative of the tissue," Gaston says. There is enough material in the print for real-time PCR for hundreds of markers. Tissue printing may also prove to be useful in very large specimens, where there are many tiny tumors, and in lymph node biopsies. The techniques are easy to learn and are beginning to be applied to other cancers (e.g., breast cancer). "It's important to establish that you don't damage the specimen in each new context," Gaston observes. "Molecular markers are moving into practice, but it takes a while to know what the markers are telling you, which markers will be most useful in the clinical course of disease. The pharmaceutical industry will be interested in licensing markers when things start to pan out."

Future Directions

Gaston sees new gene markers starting to move into clinical practice where initially they may be useful when standard histochemistry doesn't give a definitive answer and to help determine if a patient will respond to a particular therapy. Even as molecular diagnostics move into smaller settings, Gulley believes there will always be a role for centralized laboratories for more complicated test interpretations. Most university hospitals serve mainly their own patients, and there continues to be a need for reference laboratories for tests not offered at university hospitals. Array technology shows great promise, she observes, but cautions that many quality assurance issues need to be worked out before clinical laboratories will routinely use them.

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