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Guest Editorial

The Evolution of Molecular Genetic Pathology

Advancing 20th-Century Diagnostic Methods into Potent Tools for the New Millennium

A fortuitous convergence of advancements in the past decade has led to an explosion of new research in molecular pathology with consequent dramatic applications to diagnosis, prognosis, and therapeutics, as well as a clearer concept of the molecular pathogenesis of diseases. The Human Genome Project not only provided the impetus for advances in technology but also stimulated the provision of funds for innovative research. Simultaneously, improvements in information technology have led to a new paradigm, eloquently and succinctly expressed by award-winning columnist and author Thomas Friedman in 2005 (*http://www.thomaslfriedman.com*) that "The world is flat." With the fruition of the Human Genome Project, unexpected connections between diverse research avenues have been made, and scientists studying assumedly unrelated topics now discover on an almost daily basis that they have been studying complementary parts of the same conundrum.

The aforementioned events have been mirrored in the content and growth of *The Journal of Molecular Diagnostics* during its first 10 years. When the *Journal* was a mere idea back in 1996, infectious diseases and hematological disorders were the major areas of molecular diagnostic application. Only the most prevalent inherited disorders and a paucity of solid and soft tissue tumors were in the realm of the molecular diagnostician. The first issue of the *JMD* in November 1999 included a Special Report announcing molecular genetic pathology as a new joint subspecialty of the American Board of Medical Genetics and the American Board of Pathology. To date, more than 130 individuals are certified in this relatively new subspecialty. In addition, the first issue of the *JMD* contained an Editorial and a second Special Report by the Association for Molecular Pathology (AMP) Training and Education Committee on goals and objectives for molecular pathology education in residency programs, as well as three research articles related to embedding of fixed tissues for DNA- and RNA-based genotyping, detection of minimal residual disease in rhabdomyosarcomas, and fluorescence *in situ* hybridization (FISH) in lung cancer. Compare those first 37 pages of journal content to the diversity and sophistication of the 76 pages in the current issue of *JMD*: a Review on parallel sequencing in clinical diagnostics, two Consultations in Molecular Diagnostics (cystic fibrosis and Gilbert's syndrome), a Technical Advance (detection of *FMR*1), five research articles (prosthetic joint infection, detection of *HER2* in breast cancer by silver *in situ* hybridization (SISH), gene rearrangements in lymphomas, array-based analysis of microRNA in formalin-fixed, paraffin-embedded human tissue samples, and a rapid screening assay for *KRAS* mutations), and a related Commentary on *KRAS*. Surely it can be agreed that during the past decade, advances in molecular pathology have morphed our parents' and mentors' diagnostic capability into a potent and powerful resource for the betterment of mankind.

While such retrospection and comparative examination provide a historical perspective to where molecular diagnostics exists today, it is the future of the field that holds significant promise for even greater improvements in health and the continued evolution of the *JMD*. Included below are forward outlooks and insights provided by the Associate Editors of the *JMD* in their respective areas of specialization.

Molecular Hematopathology: Excitement, Bewilderment, and Future Promise

Hematopathology has a proud history of being at the vanguard of the application of molecular technologies to clinical laboratory diagnostics, with such pioneering contributions as the application of antigen receptor (immunoglobulin and T-cell receptor) gene rearrangements to the characterization of lymphoid malignancies. Cumbersome and limited Southern blot approaches have been succeeded by polymerase chain reaction (PCR)-based approaches. However, the developmental flurry of nascent amplification-dependent diagnostic assays came at a price, particularly the lack of standardization and inabil-

Supplemental material for this article can be found on *http://jmd.amjpathol.org*.

ity to meaningfully compare data between laboratories. The rigorous generation of standardized protocols for these assays has been most welcome, and we look forward to the development and adoption of standardized diagnostic, prognostic, and monitoring assays.

The application of microarrays in the past decade to hematological malignancies has been accompanied by excitement, bewilderment, and confusion. It has been exciting to witness the discovery of new genetic pathways in cancer biology, which has fostered tremendous insights in our understanding of the genomic and cellular biology of leukemias and lymphomas, allowing for more robust classification, prognostication, and therapy. However, this has been accompanied by concern that such approaches might supplant conventional (morphological and immunophenotypic) tools, and even hematopathologists themselves. This is unlikely to occur, since these technologies are best viewed as discovery tools, with the new insights we uncover used by "traditional" hematopathologists, who are most adeptly positioned to harness their power. Another concern pertains to issues of lack of validation and reproducibility of some microarray studies; molecular hematopathologists will play an integral role in resolving these issues, ensuring their judicious application to diagnostics.

An additional area of new knowledge has been in the discovery of the molecular underpinnings of neoplasms that lack recurrent (or any) conventional cytogenetic abnormalities; thus, the area of molecular characterization of, for example, karyotypically normal acute myeloid leukemia has been and should continue to be an area of growth in the meaningful classification of these neoplasms. The increasingly important arena of monitoring patients with leukemia and lymphoma by quantitative approaches has dramatically reshaped concepts of remission and led to superior milestones to gauge responses, and indeed adjustments, to therapy, with the therapy sometimes targeting a specific, and often disease-defining, molecular abnormality.

The application of functional genomics, such as by using RNAi screens, will lead to the identification of novel genetic defects. Similarly, the adoption of array comparative genomic hybridization and single nucleotide polymorphism arrays will continue to refine our abilities to characterize complex neoplasms in a more sophisticated matter. The field of epigenomics is also poised to grow, as we unravel transcriptional control defects in leukemia and lymphoma, allowing for the development of novel approaches to molecular diagnostics and therapeutics.

Molecular Testing for Microbiology

Molecular testing is now well integrated in the clinical microbiology laboratory and for some pathogens has revolutionized the diagnosis and management of infectious diseases. Molecular methods were initially adapted for slow-growing pathogens, such as viruses, and those that required specialized methods or media. One of the early success stories for the power of molecular detection was in the diagnosis of herpes simplex virus (HSV)-associated encephalitis. In the mid-1990's testing for HSV DNA in cerebral spinal fluid proved to be a sensitive and specific alternative to brain biopsy, the previous gold standard for establishing this diagnosis. This was shortly followed by US Food and Drug Administration approval of the first molecular microbiology test, which was used for the detection of *Chlamydia trachomatis* from genital specimens. With these events laboratorians and clinicians developed an appreciation that this new technology was a sensitive, rapid, and technically easy alternative to conventional culture methods.

These early tests relied on PCR, but other amplification methods, such as branched DNA and transcription-mediated amplification, also came into widespread use. As technology evolved it became possible to quantify the amount of virus directly from clinical specimens. Measuring HIV-1 RNA in plasma became the first quantitative test that was widely used clinically, allowing the individual management of patients in a manner that was previously not possible. The use of viral load testing progressed and has been applied to the management of hepatitis C virus (HCV) infections, as well as cytomegalovirus, Epstein-Barr virus, and BK virus infections in bone marrow and solid organ transplant recipients. As the clinical applications of molecular testing expanded, there were also major advances in technologies, most notably the development of real-time methods that enabled the simultaneous amplification and detection of target nucleic acid. These methods reduced the turnaround time for testing to a few hours and essentially eliminated the risk of carryover contamination. Another important advantage for viral load testing was the 6 to 7 $\log_{10}$ linear range of real-time assays. All of these factors have contributed to the rapid adaptation of real-time methods for routine use in the clinical laboratory.

Advances in technology continue, and there are now systems available, and others under development, that fully automate all steps of testing including nucleic acid extraction, amplification, and detection. The development of these systems has progressed along two paths. There are large, high-throughput automated systems that are designed to increase efficiency for laboratories that perform high-volume testing for HIV-1 and HCV viral load as well as the detection of *C. trachomatis* and *Neisseria gonorrhoeae*. There are also small, simple instruments that allow addition of the primary specimen directly into a cartridge or device with reporting of results within a few hours. These random access instruments have on-board controls and do not require highly trained technologists. The availability of these types of testing systems will undoubtedly bring molecular testing into more laboratories and launch the possibility of near patient testing or point-of-care testing for the diagnosis and management of infectious diseases.

Although there has been tremendous progress in molecular microbiology testing, challenges remain. Currently the majority of clinical laboratories do not perform molecular microbiology testing. To increase access to these important tests, simple platforms with a broad test menu are needed. There are molecular tests for the identification of some bacterial pathogens directly from a clinical

specimen, most notably methicillin-resistant *Staphylococcus aureus*. Moreover, simple sequencing methods and comprehensive databases are now available and are routinely used in some referral and large clinical laboratories for the identification of bacteria, mycobacteria, fungi, and parasites that are not easily identified using conventional methods. Currently, molecular methods have not replaced standard culture methods for routine bacterial identification and susceptibility testing, and this is viewed by some as the next big challenge. For molecular testing to replace the currently used culture-based methods, advances in both technology and our understanding of the genetics of pathogen resistance are needed, if we are to move from phenotypic to genotypic methods for susceptibility testing. Although there are skeptics who feel this is not possible, with the advances in molecular microbiology that have occurred in the past decade, the potential for the next decade appears limitless.

Solid Tumor Molecular Diagnostics

Ten years ago, solid tumor molecular diagnostics was a relatively limited area with few established assays, notably *HER2* FISH in breast cancer, translocation testing in sarcomas, and perhaps microsatellite instability testing in colorectal cancer. By the midpoint of the past decade, a massive expansion of solid tumor molecular diagnostics was already on the horizon, with the 2002 discovery of activating *BRAF* mutations in several cancer types and of activating *EGFR* mutations in lung adenocarcinomas in 2004 (for further reading, see Supplemental Material at *http://jmd.amjpathol.org*). As these mutations, especially the latter, are linked to sensitivity to specific new targeted agents, and given the recent observation of the value of *KRAS* mutations as predictors of resistance to EGFR-targeted therapies in lung and colon cancers, the volume of clinical testing in this area has grown rapidly, and this growth is set to continue on a near-exponential trajectory. (See Commentary by Associate Editor Marc Ladanyi in this issue for more perspective on the predictive potential of monitoring *KRAS* mutations.) The relatively genteel time course of the development of *HER2* FISH as a marker for treatment selection in breast cancer is being tremendously compressed in the case of these new discoveries. This places new stresses on molecular diagnostic laboratories as they are asked to develop and validate the corresponding molecular predictive assays quickly and for an ever widening range of mutations. Given the size of the solid tumor oncology market, this testing area is also at risk for novel types of pressure from pharmaceutical companies, as the choice of predictive assay can in some cases change the potential market for a given targeted agent by a factor of 2 or 3 or more.

Inherited Diseases: Genetic Testing—It's Not Just about Diagnosis

Ten years ago, the Secretary's Advisory Committee on Genetic Testing was formed, CNN announced that the future of genetic testing had arrived, the US Federal government passed laws barring large health insurers or employers from using genetic testing to discriminate against individuals, and the Human Genome Project released a map of the human genome. That map included 30,000 genes, which were estimated at the time to represent one-third of the total human genes. Full-scale human genome sequencing quickly ensued. In 2001, the first draft sequence of the human genome was announced by both the National Institutes of Health (NIH) Human Genome Project and the Celera Genomics groups. The total number of human genes was revised as 30,000 to 35,000, with a subsequent revision of 20,000 to 25,000 when the Human Genome Project was completed in 2003, a year that also marked the 50th anniversary of Watson and Crick's description of the double helix structure of DNA. The first part of this century saw an explosion of completed genome sequences of both simple and complex organisms including yeast, Archaea, *Escherichia coli*, *Mycobacterium tuberculosis*, *Caenorhabditis elegans*, *Drosophila*, *Arabidopsis*, rat, chicken, chimpanzee, trypanosomatid, dog, rhesus macaque, and platypus.

Ten years ago the only private company talking about DNA sequencing was Celera, using a shotgun sequencing strategy that complemented the mapping approach of the NIH Human Genome Project. Now in 2008, we have 19 genome projects including the cancer genome and microbiome. We strive to take the foundation that has been laid in DNA sequence and understand the functional organization and regulation of our genome sequence. We now know that the human genome consists of probably only 20,000 genes, but there is extensive "multitasking" (to coin the term used by Dr. Francis Collins, former director of the National Human Genome Research Institute) of expression in our genome with alternate transcripts and processing contributing to genetic diversity. Identifying the switches, triggers, exposures, and interactions that control these proteins are the next challenges for geneticists.

What will we do with our new genetic information and disease risk factors? We are at the beginning of personalized medicine with gene-targeted therapies but not yet realizing effective gene therapy. Expanded newborn screening, pharmacogenomics, and nutrigenomics hold promise to help us identify and perhaps attenuate our individual genetic risks for disease susceptibility. "The right drug at the right dose and the right time" is the aim and perhaps the modern restatement of *primum non nocere* (first, do no harm). In the US we have recently passed the Genetic Information Nondiscrimination Act but also have personal genome companies marketing directly to the public with vague promises of increased understanding of genetics. The implications of our newly found genetic knowledge continue to ripple through all aspects of our society.

Ten to 15 years from now medicine will be quite different as personalized medicine develops both diagnostics and targeted treatments. With application of more emphasis on disease prevention and managing exposures from our environment, diet, or lifestyle, we may significantly influence the clinical outcome of our genetic risk. As laboratory professionals we must engage in active

education with our physician and health care colleagues to aid the integration of these advances into routine patient care decisions. We are not limited by lack of good ideas or methods or dedicated individuals but by decreasing financial resources just at the time knowledge is booming. With the recent passing of Victor McKusick, the father of human genetics, let us renew our efforts to strive for responsible application of our understanding of our genomes to improve the diagnosis and treatment of genetic disorders including equal access to medical care for all of our citizens as well as to all global populations in need.

Closing Remarks

As we move further into the new millennium, the usefulness and ease of implementation of molecular testing will no doubt continue expanding. The evolution of molecular diagnostics (as well as molecular prognostics and therapeutics) will continue to improve health care and quality of life into the future, and *The Journal of Molecular Diagnostics* will continue to lead the way in molecular medicine and analysis of new assay developments.

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