MOLECULAR ONCOLOGY 6 (2012) 177–181

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Molecular pathology☆

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ARTICLE INFO

Article history:
Received 24 February 2012
Accepted 27 February 2012
Available online 23 March 2012

Keywords:
Molecular pathology
Biomarkers
Genomics
Gene sequencing

ABSTRACT

Molecular pathology as applied to neoplasia is a rapidly expanding component of the discipline of pathology that uses molecular biology tools in addition to conventional morphologic, immunohistochemical and chemical analyses of abnormalities in tissues and cells to understand the etiology and pathogenesis of tumors, establish their diagnosis, and contribute to prognostication and therapeutic decisions for cancer patient care. Biomarkers are a fundamental component of personalized cancer care, and the discipline of molecular pathology therefore contributes throughout the continuum from biomarker research to use in standard-of-care personalized cancer therapy. This brief review addresses some of the specific roles of molecular pathology in that continuum.

1. Definitions and context

Personalized cancer medicine and other similar terms are now widely used but have a large number of definitions and connotations depending upon the context of use, ranging from the generic to the highly specific. At the generic end of the spectrum, personalization is all-encompassing and is the tailoring of all aspects of cancer care to the spectrum of characteristics of each individual patient (President's Council of Advisors on Science and Technology, 2008). A far more specific definition is the use of genetic biomarkers and/or pharmacogenomic testing to tailor an individual's preventative care or therapy for cancer (Burke and Zmmern, 2004).

Personalized cancer therapy is influenced by the characteristics of the patient's tumor, genetic constitution, and environmental exposures and lifestyle, as well as the characteristics of the treatment modalities including surgery, radiation therapy, and chemotherapy. In the case of chemotherapy, these characteristics can be referred to as pharmacogenomics based on tumor alterations, pharmacogenetics based on patient germline characteristics, pharmaco-epidemiology based upon exposures and lifestyle, and pharmacokinetics/pharmacodynamics based upon the characteristics of the therapeutic agents. Molecular pathology has important roles in characterizing both the cancer and the patient who has that tumor for use in clinical management.

Molecular pathology as applied to neoplasia is a rapidly expanding component of the discipline of pathology that uses molecular biology tools in addition to conventional morphologic, immunohistochemical and chemical analyses of abnormalities in tissues and cells to understand the etiology and pathogenesis of tumors, establish their diagnosis, and contribute to prognostication and therapeutic decisions for cancer patient care. The discipline of molecular pathology thus contributes throughout the continuum of biomarker research leading to incorporation into standard-of-care personalized cancer therapy. This continuum includes discovery; assessment of viability and feasibility; planning; development, integration and verification; validation including clinical trials; launch readiness and release; application in patient care, and subsequent post-implementation evaluation of performance (Phillips et al., 2006). Therapeutic agent development also utilizes molecular pathology in research and clinical approaches to target identification, attempts to distinguish

☆ For special Molecular Oncology issue organized by Ulrik Ringborg, MD, PhD.

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1574-7891/$ – see front matter © 2012 Published by Elsevier B.V. on behalf of Federation of European Biochemical Societies.
doi:10.1016/j.molonc.2012.02.007

between driver alterations that are pathogenic and passenger alterations that do not directly control tumor behavior, assessment of the effects of agents on reputed targets and resultant downstream responses in pathways, identification of biomarkers for agent response and resistance, and rational selection of combinations of therapeutic agents.

2. Clinical biomarker development

The processes for bringing a biomarker into clinical use involve many sequential and parallel steps. Initial data must be obtained to support the investment of resources to develop a biomarker. Definition of the intended clinical use is a key first step (Brock, 2012), and the selection of the patient populations and specimens to match the intended clinical use is essential to providing high-quality data. The biomarker assay must be validated at the laboratory level and carefully quality controlled including use of reference materials and proficiency-tested personnel (Holden et al., 2011) in order to provide the basis for clinical validation. Study design is important with sufficient sample size for statistical power and the ability to demonstrate that a biomarker test improves clinical outcome. The Institute of Medicine of the National Academies in the United States released a comprehensive report with recommendations on omics assays (Micheel et al., 2012).

3. Laboratory assay methodologies

The laboratory methodologies and technologies available for use in molecular pathology are extensive and address a wide variety of analytes including chromosomal DNA, mitochondrial DNA, messenger RNA, non-coding RNA that includes microRNAs, proteins, and lipids (Louis et al., 2011; McDermott et al., 2011). As the laboratory-based "-omics" including genomics, transcriptomics, proteomics, metabolomics, etc., have emerged, bioinformatics and biostatistics have moved front and center in the analyses and interpretation of the resulting massive data sets (Vickers, 2008).

DNA evaluation includes a variety of sequencing approaches ranging from directed characterization of individual genes through Sanger sequencing and pyrosequencing to broad-scale "next generation" sequencing that can be applied across all exomes or the whole genome (McDermott et al., 2011). The availability of various methodologies for DNA sequencing provides the opportunity to employ a number of approaches to tumor characterization. These range from targeted sequencing of specific individual genes (Sanger and pyrosequencing) to whole genome sequencing that addresses the breadth of somatic mutations in tumor DNA in comparison to germline sequences, and germline mutations and polymorphisms compared to "normal" sequences. Intermediate to these methods is the evaluation of the transcribed portion of the genome through whole exome sequencing. These methodologies have advantages and disadvantages, with inverse relationships between the amount of information that is obtained and the ease of analysis.

Identification of the most effective sequencing strategies is important as these technologies move into clinical utilization. Multiplex techniques can identify the whole range of mutations in tumors and define changes in specific biologic pathways that have potential to be targeted by therapeutic agents. A major challenge in clinical usage is the reporting of results in a meaningful fashion (Louis et al., 2011) and determining the efficacy of agents that are selected on the basis of mutation, including novel combinations of agents for which toxicologic data and drug interaction information may not be available.

These newer methodologies have led to the recognition of the remarkable extent of nucleotide sequence variation in the germline genome and in the somatic abnormalities in cancers. The methodological advances identified a small subset of cancers with hypermutation characterized by extremely frequent sequence alterations as compared to germline sequence (Stratton et al., 2009; Kumar et al., 2011). Sequencing can also address copy number variation in the form of amplifications and deletions, as can older techniques such as fluorescent in situ hybridization (FISH) and analysis for loss of heterozygosity in polymorphic areas of the genome. Rearrangements of chromosomal structure in the form of translocations and inversions occur and can be detected by cytogenetics and by sequencing. Methylation of CpG islands in the promoters of many genes that suppresses transcription and alter chromosomal structure can be analyzed by sequencing after bisulfite conversion and by high-throughput methodologies.

RNA evaluation addresses two major groups of analytes: messenger RNA (mRNA) that is translated into proteins and the much more prevalent non-coding RNAs that include microRNAs (miRNAs) responsible for regulation of gene expression. Individual genes can be assessed by reverse transcription into DNA and polymerase chain reaction for quantitation (QRT-PCR), but the transcriptome can also be evaluated by gene expression profiling with hybridization to chips that are spotted with cDNA or by RNA sequencing.

Protein analysis has been a mainstay of molecular pathology for decades through the application of immunohistochemistry. Recent advances in proteomic methodologies now permit broad and detailed unbiased characterization of proteins, but antibody-based technology applied to reverse phase protein arrays can also address simultaneously the alterations in large numbers of selected proteins, including post-translational modifications such as phosphorylation. Recent technical advances have led to the ability to assess lipids, including lipidomic methodologies, and metabolomics can characterize a wide variety of metabolic products.

The large number of available methodologies is daunting, but available evidence on the importance of individual types of alterations suggests that many different technologies will be needed for clinical applications. For example, sequencing of KRAS has become standard-of-care for patients with colorectal cancer eligible for treatment with antibodies to epidermal growth factor, copy number variation evaluation of Her2 for breast cancer and trastuzumab, translocations involving Bcr and Abl in chronic myelogenous leukemia for imatinib and EML4 and Alk in lung cancer for crizotinab, methylation of MGMT for temazolamide for glioblastoma multiforme, and gene expression panels for recurrence score in breast cancer. As multiple therapeutic options associated with

biomarkers become available in various tumor types, multiplex testing must expand for cost-effective evaluation of tumor in patients with multiple therapeutic options.

4. Co-alterations and pathway dysregulation

The single greatest challenge for molecular pathology at present is the integration of the many sources of data into meaningful and actionable knowledge that can be applied to improve patient outcomes. The complexity of the alterations in individual cancer cells multiplied by the intratumoral heterogeneity among the population of cancer cells within a tumor along with the temporal interactions of the cancer cells with their changing microenvironment populated by stromal and inflammatory cells and a plethora of molecules from various sources is truly staggering. This complexity has long been apparent when individual methodologies were applied to molecular pathology, but the comprehensive approaches such as The Cancer Genome Atlas and International Cancer Genome Consortium (The Cancer Genome Atlas Research Network, 2008; Cancer Genome Atlas Research Network, 2011) have pointed out the incredible frequency of co-alterations, the extent of dysregulation of numerous cellular pathways, and the crosstalk among the pathways. Intertumoral heterogeneity is obvious, and it is now glaringly apparent that no two patients ever have molecularly identical tumors. The hope for clinical application of molecular pathology is that the crucial alterations in pathways, rather than the details of how each pathway is altered, can be characterized eventually to identify actionable biomarkers.

5. Decision-making on biomarker analysis approaches

Criteria usable in decisions regarding the readiness of a biomarker assay for research and/or clinical use have not been well established despite repeated attempts made to determine strength or levels of evidence for decision-making. Green and Byar presented in 1984 an eight point scale for determining treatment efficacy based on analysis of existing data sets. Their categories ranged from anecdotal case reports to confirmed randomized controlled clinical trials (Green and Byar, 1984). Hayes et al. reported in 1996 the much more broadly applicable Tumor Marker Utility Grading System (TMUGS) that addresses biomarker use in determining risk, screening, differential diagnosis, predicting relapse or progression of primary or metastatic disease, predicting response to therapy, and monitoring the course of disease to detect relapse or follow detectable disease. The system included a utility score and level of evidence number for process and endpoint association with a biologic as well as with indicators of patient outcome (survival, disease-free survival, quality of life, and cost of care). The comprehensive nature of the system is also its deterrent to use due to the complexity. Lassere and colleagues reported in 2007 a schema that is based on target, study design, statistical strength, and penalties (Lassere et al., 2007) that has not gained wide acceptance. In the absence of quantitative approaches, opinion of experts on the breadth and depth of evidence continue to guide most decision-making, often through guidelines issued by professional organizations.

Despite the weak evidence-based and haphazard approaches, numerous markers have moved into routine usage. Recent literature reviews have summarized the current status of clinical molecular testing for a variety of common cancers, e.g. breast, colon, lung, pancreatic, and thyroid tumors, sarcomas, melanomas, and tumors of uncertain origin (Igbokwe and Lopez-Terrada, 2011).

6. Pre-analytic challenges for biomarker development and use

6.1. *Complexity of molecular changes in neoplasms*

As described above, the complexity of the molecular changes in neoplasms is the most formidable challenge to biomarker development. This situation is due to the large number of opportunities that are afforded, uncertainty about the drivers, non-static temporal changes, reactive pathways, and intra- and intertumoral heterogeneity. Molecular classifications of tumors have potential to identify entities with clinical importance, if clinical associations can be identified and validated.

6.2. *Specimen acquisition and qualification*

An essential component of molecular pathology is the provision of high-quality tissue specimens for both research and clinical care activities. Great emphasis has been placed on research biorepositories with biospecimens for translational research (National Cancer Institute, 2011). The importance of population-based specimen acquisition and biospecimens from patients enrolled in clinical trials has increased, as successful studies with specimens from these two sources provide high levels and broad breadth of evidence for moving biomarkers along the pathway to clinical application. The methodologies for molecular pathology characterization put new emphasis on frozen and appropriately stabilized fixed tissue, in addition to the routine formalin-fixed paraffin embedded specimens generally available in pathology laboratories. This new emphasis is necessitated by the analytic methods that cannot address, or address poorly, even modestly degraded specimens. A change in laboratory approaches in all pathology laboratories is needed to provide the high-quality specimens, as buffers/fixatives for preservation of labile analytes (messenger RNA, proteins, phosphoproteins, etc.) are not generally available. The ability to down-scale tissue quantity is also an issue, as smaller specimens derived from interventional radiology procedures and endoscopic biopsies will be the only appropriate tissue available in many efforts directed at personalized cancer therapy. Amplification of some analytes may increase the amount of material but may be unsuitable due to perturbation from the native state. Qualification of specimens for analysis of intact tumor is especially problematical with destructive routine histopathology, and real-time non-destructive evaluation methodologies are needed to provide the maximum amount of available high-quality tissue.

Fit-for-purpose selection of tissue sources is important for analytes of interest that vary in their characteristics. Elapsed time from interruption of blood supply to stabilization of the specimen is crucial for labile analytes. The much longer time interval between specimen acquisition and clinical decision can also be important, as the decision to analyze primary tumor, synchronous or metachronous metastatic tumor, or recurrent tumor before or after prior therapy has the potential to influence the utility of the laboratory analysis results. Invasive procedures, often by interventional radiologists, are needed to acquire tissue from common metastatic sites such as the liver and lungs. There is therefore great interest in using circulating tumor cells and nucleic acids because of their easy access by phlebotomy, availability contemporaneous with need for therapeutic decisions, and potential for sequential analyses, but the representativeness of a patient's tumor is an issue that remains to be addressed.

Biorepositories have extensive responsibilities in tracking consent, addressing sample collection requests, determining the time of collection and processing, and maintaining inventory control with location of specimens. In addition, sample retrieval, checkout, and distribution must be managed. Many biorepositories also annotate the specimens with clinical information from the patients who provide the specimens. Standard operating procedures for specimens, governance documentation, access and prioritization processes, review of applications for use, and administrative interactions are needed. The National Cancer Institute's Best Practices for Biospecimen Resources (National Cancer Institute, 2011) provides a comprehensive reference, and other organizations have guidelines and/or accrediting activities for clinical specimen biorepositories in preparation or available.

6.3. *Intratumoral heterogeneity: tumor and stroma*

Although most cancers are clonal proliferations, evolution of subpopulations (subclones) of tumor cells is well-known to occur during tumor growth. The size and topographical distribution of the abnormal subpopulations in tumors are as yet poorly studied. It is unclear if small subpopulations, that in turn may be difficult to detect with some analytic methodologies, are important in therapeutic choices.

In addition to the characteristics of the tumor cell population, the potential effects of stroma composed of non-neoplastic cells must be addressed in specimens for biomarker analysis. The stroma is generally considered a contaminant of the tumor cells that complicates analyses by contributing non-neoplastic material into the analysis material, but it is now recognized that tumor microenvironment is a key element. Analyses may need to address stromal cells as well as tumor cells to achieve a complete understanding of tumor characteristics.

7. Biomarkers in clinical trials

An important goal in the application of biomarkers is to make better use of currently available therapeutic agents by finding subsets of responders, as well as contribute to the development of new agents in clinical trials. The development of predictive biomarkers associated with chemotherapeutic and targeted agents is particularly difficult and is best done in the setting of clinical trials with carefully selected and monitored patient cohorts. The clinical challenges include the low response rates for many agents, the use of combination chemotherapy that makes separation of biomarkers for individual agents difficult, differences in dosage and timing of drug administration that may affect the informative timepoints for biomarker assessment, and the range of biomarkers and methodologies that are available. A practical problem with combination therapy is the origin of agents from different pharmaceutical and biotechnology companies with the associated intellectual property issues. Biology is also important, as sensitivity and resistance of tumor cells are complex biological processes. The mechanisms of effects of different therapeutic agents influence this complexity, e.g. cytostatic as compared to cytotoxic agents. Trafficking of signaling through pathways is at an early stage of understanding, and the response capabilities of tumors after perturbation by therapeutic agents is not predictable from baseline status in many instances. Plasticity of the cancer cell population, including the acquisition of cancer stem cell characteristics after exposure to agents, remains to be explained and addressed.

Resources must be in place for successful biomarker development in clinical trials, and these include tissue repositories; data management, bioinformatics and biostatistics; sources of funding; and timing of biomarker development relative to therapeutic agent development. Multiple assays must be used for assignment of patients to arms of a trial with different agents when each agent has an associated biomarker, but in order to be cost-effective and conserve valuable tumor specimens, multiplex assays (Roychowdhury et al., 2011) with regulatory compliance are needed. Gene patents influence the ability of clinical laboratories to develop assays, including the needed multiplex assays, because patent rights can lead to requirements for licensing or to submit all testing to a commercial laboratory owned by the patent holder (Chen et al., 2010). At present, the status of gene patents in the United States remains to be determined by the Supreme Court.

The highest level of evidence for clinical use of a biomarker comes from an integral marker or integrated marker adaptive randomization clinical trial in which the biomarker results determine assignment to a therapeutic arm (Hayes et al., 1996). These challenging trials must meet regulatory compliance for performance of the assay, and use of a central laboratory has major advantages due to the concentration of expertise, instrumentation, and economies of scale. As biomarkers become established, distributed laboratories are far more commonly used. The source of funding to develop biomarkers is often problematical due to contractual issues. Payers in clinical trials include the trial sponsors, the National Cancer Institute, and third party payers, and appropriate distribution of cost is often difficult.

8. Standard-of-care application of biomarkers

The approaches to appropriate use of biomarkers in routine patient care are unsettled at present. On one hand, the level of confidence in the performance of a biomarker for

therapeutics must be sufficiently high for physicians to decide to give or withhold drugs from a particular patient. On the other hand, the promises and cache of novel biomarkers derived from state-of-the art science have great attraction for their use. Direct-to-patient and direct-to-physician marketing of unproven biomarkers is commonplace and often done in the absence of peer-reviewed publication of laboratory validation of the methodologies, let along clinical validation of the utility of the biomarkers in managing patients.

A variety of providers of molecular diagnostics is available, including hospital laboratories, large and small reference laboratories, and diagnostic assay companies. The current regulatory environment generally addresses the quality of the laboratory analysis of biomarkers. In most countries, regulatory agencies have major impact on the utilization of molecular pathology in clinical applications. In the United States, the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) govern the accreditation of laboratories that provide laboratory results for use in patient care. In addition, a wide variety of professional organizations have published guidelines and recommendations for use of biomarkers, and various organizations have specific requirements governing the usage of laboratory-developed tests (LDTs). As assays have become increasingly complex, involving informatics algorithms as well as multiplex testing, quality control/quality assurance becomes an increasing challenge. In addition, the Food and Drug Administration (FDA) in the United States has established specific companion diagnostics based on specific manufacturer's assays that are linked to the approval of associated therapeutic agents (Brock, 2012).

The circumstances around reimbursement for molecular pathology in the clinical setting require further development. The criteria and processes for determining a molecular diagnostic test is "standard-of-care" in a specific clinical setting have not been established and are inconsistent among payers. Development of documentation of medical necessity and billing compliance as well as post-implementation evaluation of outcomes and clinical effectiveness remain poorly addressed.

9. Future directions

Molecular pathology will continue to grow in importance as the continuum from biomarker research to clinical applications in personalized cancer therapy continues to expand and become more robust. The broad areas for biomarker application to risk identification, screening, surveillance, diagnosis, prognostication, prediction of response and resistance, and monitoring for response and recurrence are well-known, but the use-cases for specific biomarkers need to be established. Ideally, future biomarker development should be prioritized for greatest impact on improving patient care with rapid laboratory method validation, clinical validation, and subsequent access for patients. Technological advances will continue to provide new methodologies to be evaluated and applied in molecular pathology laboratories that need to be resourced to acquire appropriate material from patients, develop and provide assays, provide quality control/quality assurance, and meet regulatory and reimbursement requirements.

REFERENCES

Brock, T.K., 2012. Diagnostic, prognostic, and predictive: the dynamic role of molecular technology in personalized medicine. Am. Lab., 21–25. January.

Burke, W., Zmmern, R.L., 2004. Ensuring the appropriate use of genetic tests. Nat. Rev. Genet. 5, 955–959.

Cancer Genome Atlas Research Network, 2011. Integrated genomic analysis of ovarian carcinoma. Nature 474, 609–615.

Chen, Q., Ye, Z., Lin, S.-C., et al., 2010. Recent patents and advances in genomic biomarker discovery for colorectal cancers. Recent Patents on DNA & Gene Sequences 4, 86–93.

Micheel, C.M., Nass, S.J., Omenn, G.S. (Eds.), 2012. Evolution of Translational Omics. Lessons Learned and the Path Forward. Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials. Institute of Medicine of the National Academies. The National Academies Press, Washington, D.C. March 23, 2012. www.nap.edu.

Green, S.B., Byar, D.P., 1984. Using observational data from registries to compare treatments: the fallacy of omnimetrics. Stat. Med. 3, 361–370.

Hayes, D.F., et al., 1996. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J. Natl. Cancer Inst. 88, 1456–1466.

Holden, M.J., Madej, R.M., Minor, P., et al., 2011. Harmonization through reference materials, documentary standards and proficiency testing. Expert Rev. Mol. Diagn. 11, 741–755.

Igbokwe, A., Lopez-Terrada, D.H., 2011. Molecular testing of solid tumors. Arch. Patho; Lab. Med. 135, 67–82.

Kumar, A., et al., 2011. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc. Natl. Acad. Sci. USA 108, 7087–17092.

Lassere, M.N., et al., 2007. Definitions and validation criteria for biomarkers and surrogate endpoints: development and testing of a quantitative hierarchical levels of evidence schema. J. Rheumatol. 34, 607–615.

Louis, D.N., Virgin, H.W., Asa, S.L., 2011. Next-generation pathology and laboratory medicine. Arch Pathol. Lab. Med. 135, 1531–1532.

McDermott, U.M., Downing, J.R., Stratton, M.R., 2011. Genomics and the continuum of cancer care. N. Engl. J. Med. 364, 340–350.

National Cancer Institute, 2011. Best Practices for Biospecimen Resources.

Phillips, K.A., Van Bebber, S., Issa, A.M., 2006. Diagnostics and biomarker development: priming the pipeline. Nat. Rev. Drug Discov. 5, 463–469.

President's Council of Advisors on Science and Technology, 2008. Priorities for Personalized Medicine, p. 13.

Roychowdhury, S., et al., 2011. Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci. Translational Med. 3, 1–10.

Stratton, M.R., Campbell, P.J., Futreal, P.A., 2009. The cancer genome. Nature 458, 719–724.

The Cancer Genome Atlas Research Network, 2008. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455, 1061–1068.

Vickers, A.J., 2008. Decision analysis for the evaluation of diagnostic tests, prediction models and molecular markers. Am. Stat. 62, 314–320.