Role of proteomics in personalized medicine

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"From the perspective of diagnostics, the transition from primarily morphological assessments into a detailed, real-time molecular diagnosis for each patient would represent a major improvement in patient care."

Cancer diagnosis and therapy is at the forefront of the personalized medicine revolution. A personalized approach to therapy guided by molecular profiling of cancer tissue is the major transformation depicted in the following vignettes.

Vignette 1: current practice

A 45-year-old female presents at a clinic with a complaint of shortness of breath. After a thorough cardiovascular evaluation that reveals no evidence of heart disease, a computed tomography (CT) scan of the thorax reveals several large nodules in the right lung. An endoscopically-obtained tissue biopsy is taken and fixed in formalin for morphological analysis by a pathologist. Under a microscope, the pathologist examines the tissue section that has been stained with hematoxylin and eosin. Based on experience, the pathologist classifies the tissue as a poorly differentiated adenocarcinoma of the lung. A subsequent staging imaging study is performed revealing likely significant nodal involvement. Based on years of clinical correlations and clinical trials, this patient begins a treatment regimen including chemotherapy. The oncologist communicates to the patient that a range of patient responses to the proposed chemotherapies has been observed, from good to poor response. Patient concerns over the side effects of therapy are balanced against the hope of a good response. At present, based on morphological assessments, the type of response cannot be predicted.

Vignette 2: personalized medicine of the future

A 45-year-old female presents at a clinic with a complaint of shortness of breath. After a thorough cardiovascular evaluation that reveals no evidence of heart disease, a CT scan of the thorax reveals several large nodules in the right lung. An endoscopically-obtained tissue biopsy is taken and immediately placed in a preservative compatible with molecular profiling in addition to morphological assessment. Using laser capture microdissection, a clinical laboratory team extracts tumor cells for further study. The cells are lysed and the molecular constituents (DNA, RNA and proteins) are quantitatively analyzed by molecular profiling technologies. Derangements within the tumor cells' molecular circuitry are identified using antibody probes that detect the presence of phosphate groups on important signaling proteins established as drug targets. The pathologist prepares a protein circuit map highlighting important alterations in the molecules of the tumor cells. This not only provides diagnostic information, but also yields insight regarding the choice of targeted therapeutics that should be used. Furthermore, multiple molecules within the deranged molecular circuitry can be targeted for simultaneous combinatorial treatment, reducing side effects and minimizing the probability of resistance.

Factors driving medicine toward more personalized approaches

Cancer is often discovered when it leads to signs and symptoms that drive the patient to the physician's office. To date, the cornerstones of solid tissue cancer diagnosis focus on physical examination, imaging and pathological evaluation of biopsied tissue. However, morphological assessments of disease provide limited insight into the unique, individual molecular qualities of a particular patient's disease, the knowledge of which could dramatically alter patient treatment and response. This limitation is the driving force behind the development of personalized medicine. From the perspective of diagnostics, the transition from primarily morphological assessments into a detailed, real-time molecular diagnosis for each patient would represent a major improvement in patient care.

Although molecular diagnostics such as chromosomal analysis, detection of clonal populations by polymerase chain reaction (PCR), *in situ*

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hybridization and immunohistochemistry have been an important component of pathological diagnoses for some time, new technologies have emerged that will enable high-throughput molecular profiling of patient tissues in the future. Such molecular profiling platforms will open the way for personalized assessments of a patient's tumor cells, providing insight into the molecular processes that underlie the disease process. Hand in hand with molecularly-targeted therapies, a future is envisioned wherein patients receive therapeutics matched to their particular disease, enhancing response and limiting detrimental side effects.

Translating bench research into clinical tools

Many years of bench research have revealed some of the molecular underpinnings of carcinogenesis. These efforts have relied on systems, such as cell culture and animal studies, which attempt to model events occurring within a human patient. The identity of a rich repertoire of cancer-related molecules, including extracellular matrix molecules, cell surface molecules, metabolic enzymes and signaling proteins, among others, have emerged from these studies. The challenge now is to correlate these findings with molecular derangements linked to a particular patient's disease. Early studies using protein microarrays demonstrated that the molecular content within cancer cells taken from prostate cancer tissue is divergent from that extracted from prostate cancer cells grown in culture [2]. This finding is attributable to the inability to recapitulate the three-dimensional molecular and cellular forms that surround and comprise a tumor microenvironment using *in vitro* culturing conditions. The extracted cell lines are no longer subject to molecular stimuli found in the microenvironment of a complete tissue [1]. Translational research using human tumor tissue specimens is therefore taking a center-stage role for molecular profiling studies. Another finding that underscores the importance of studying cells extracted from human cancer tissue specimens was revealed when the proteomic content of primary and metastatic tumors was compared. In this study, divergent molecular profiles were found between primary and metastatic tumors [3]. Given the centrality of human tissue in understanding cancer pathogenesis, it is very important to develop and test technologies that provide personalized molecular information for cancer patients.

Molecular profiling

New molecular techniques, such as genomic and proteomic profiling, are revealing previously hidden information regarding cellular processes. Genomics studies have identified numerous disease susceptibility genes, potential targets for therapeutic interventions and disease-relevant expression profiles [4–6]. While genomics has opened the door to thinking about how to create systems that deliver personalized medicine, the molecular information gleaned from these approaches provides an incomplete view of disease processes. Much of the work of cellular systems is carried out by proteins, and proteins are therefore prime drug targets. Correlation of gene transcript levels and protein levels in living systems is problematic [7]. Moreover, proteins are often altered via posttranslational modifications like phosphorylation, cleavage, glycosyation and lipidation. Such post-translational changes will, by definition, be missed by genomics studies. As post-translational modifications of proteins and protein–protein interactions are important in the etiology of disease processes, it is important to detect and measure protein-related changes in patient tissues [8,9]. These challenges are key factors that have propelled molecular profiling into a postgenomics era. From the perspective of cancer diagnostics and therapy, signaling proteins are of increasing importance as biomarkers of disease and therapeutic targets. Phosphorylated proteins form complex, interrelated signaling cascades that drive behaviors associated with cancer, such as avoidance of apoptosis, migration and cell division.

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As disease-related proteins are of increasing interest and human tissue will be an essential component of personalized medicine systems, new laboratory infrastructure is required that measures low abundance protein analytes present in patient tissue specimens. While standard immunohistochemistry and tissue arrays enable protein epitopes to be detected, they are difficult to objectively score and quantification of epitopes is poor. Borrowing elements of immunohistochemical studies and tissue arrays, protein microarrays are being developed as a means to perform quantitative, precise, highly reproducible, and high-throughput analyses of proteins [10,11]

Reverse phase protein arrays probe molecular information in tissues

Reverse phase protein microarrays comprise a clinically relevant array system that enables protein analytes extracted from tumor cells within patient tissue specimens to be probed with panels of antiphosphoprotein antibodies. Cells from a tissue specimen can be isolated by laser capture microdissection of a frozen tissue section. Heterogenous protein analytes extracted from the cells are directly arrayed onto a substrate. The protein spots are then probed with antibodies that detect analytes of interest, such as phosphoproteins. Phosphoproteins can be detected at a level of less than ten cell equivalents [11–14]. This system has been applied to a number of malignancies, can be integrated into a clinical workflow and provides a mechanism to bring personalized medicine into pathology practice.

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Challenges

Personalized medicine promises to improve patient evaluations and care. However, significant changes in the healthcare delivery system are required to accommodate the new approaches that constitute personalized medicine. These changes will challenge the *status quo* and will require sector-wide cooperation and synergistic collaborations. Key challenges are discussed below.

New tissue collection systems

The current standard of care for patients includes formalin-based fixation of surgical specimens and biopsies. While this approach is adequate for morphological assessments and DNA-based molecular profiling, it is unsatisfactory for proteomic studies. New tissue collection protocols are required that preserve tissue morphology and stabilize RNA and protein molecules for transcriptional and proteomic profiling. To realize the vision and hope of personalized medicine, pathologists, surgeons, radiologists, oncologists, nurses, hospital administrative staff and patients must all be educated on the importance of tissue preservation and work together to create accepted protocols for tissue preservation.

Development & testing of molecular profiling platforms in clinical settings

Reverse phase protein microarrays coupled with laser capture microdissection is one technology that is being tested as a molecular profiling system. This and other systems must be validated in clinical laboratory settings run under College of American Pathologists–Clinical Laboratory Improvement Amendments (CAP–CLIA) guidelines.

Interdisciplinary cooperation

The move toward personalized medicine will require collaboration between basic scientists, pathologists, oncologists, bioinformaticists, epidemiologists, radiologists and bioethicists in order to generate lists of potential biomarkers and develop multicenter trials to generate consensus standards of care. The nature of personalized medicine, being linked to molecular profiling, will cause some merging among previously distinct clinical specialties. Medical specialists will need to be flexible and willing to adjust to the development of a new molecular medicine paradigm. Physician, resident and student education and training, in addition to nursing education, will need to be modified and expanded to assimilate a rapidly growing new medical discipline. The time and effort necessary to create a body of data to support an evidence-based medicine rationale for implementation of discoveries generated by personalized medicine research will be considerable. A significant advantage for personalized approaches is that more selective clinical trials could be designed in order to decrease the time and number of patients in a trial. Ethical considerations resulting from the identification of disease suspectibilities will likely emerge and mechanisms for family testing must be formulated.

Identifying patients for molecular profiling

The ability to molecularly classify tumors will necessarily lead to the following question. Which patients should be evaluated? Recent work in serum proteomics has identified a low-molecular-weight serum proteome that is being investigated for the presence of disease-related biomarkers [15,16]. The goal of this work is to generate panels of biomarkers that can be used to detect early stage, preclinical disease. Patients identified as having early-stage disease will then be sent for molecular studies. Identification of this patient population may improve patient response to therapy [17].

New technologies will meet regulatory challenges

How will regulation by the US FDA, state agencies, the Joint Commission on Accreditation of Healthcare Organizations (JCAHO), and medical specialty societies deal with the introduction of new, unique and novel biomaterials and markers? The US Congress is aware of the challenges that personalized medicine processes and procedures will introduce into the healthcare system, and has begun to address these issues by considering legislation such as the Advanced Laboratory Diagnostics Act of 2006.

Reimbursement

Molecular profiling techniques will require new billing codes and acceptance for reimbursement by government and insurers. This includes reimbursement not only of the required testing, but also the administration of expensive drugs in 'off label' settings. Will economically disadvantaged hospitals be able to offer personalized molecular profiling? Financial analytical models will have to be developed to gauge the economic impact of these new technologies.

Summary

Medicine is at the threshold of a major shift in the care of patients. Personalized molecular portraits of patient disease processes will provide unparalleled access to the molecular descriptors of disease and delineate targets for therapeutic intervention. Protein microarrays will be a key initial technology as the transition into laboratory molecular profiling is made. Significant collaboration between diverse medical specialties, hospital administrators, insurers and government is required to move beyond visionary thinking and into actual profiling of patient tissue specimens as part of a personalized medicine assessment.

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