are working to identify disease biomarkers that may detect early disease. Patients with elevated early-stage biomarkers can then be referred for molecular imaging. Joint meetings and proposals between molecular imaging specialists and pathologists could be one starting point for such collaborations.

- Encourage more hypothesis-driven science in the development and validation of surrogate markers. Develop and carry out such studies to support confidence in the surrogate marker before it transitions to clinical use.

Summary Statement

Basic research supporting molecular imaging development is flourishing as the potential benefits of these techniques in patients with a range of disease and health issues becomes apparent. The immediate challenges are to attract and train new talent from a range of scientific disciplines to bring a synergetic focus on the most crucial questions; to work collaboratively with industry, professional organizations, academia, and regulatory bodies to streamline the bench-to-bedside process, and to identify the right questions that will direct research and discovery in this rapidly expanding field.

Michael J. Welch, PhD
Chair, Basic Research Issues Session

Mathew L. Thakur, PhD
Cochair, Basic Research Issues Session

PRESENTATIONS

Oncogene Cell Differentiation/Cell Transduction

Imaging, in combination with physical examination and pathologic evaluation, is a key element of cancer diagnosis and staging. To date, cancer imaging largely focuses on determining the precise localization of the primary tumor and sites of metastatic disease. Elements of a diagnostic report include the site and size of a cancer as well as the nodal status and the presence of disease in other organs. These end points have improved information available to oncologists and refined treatment regimens. Morphologic assessments of disease, however, provide limited insight into the unique, individual 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 molecular or personalized medicine. From the perspective of molecular imaging and pathologic diagnosis, the transition from primarily a morphologic assessment into a detailed, real-time molecular diagnosis for each patient would represent a quantum leap in patient care. This would enable molecular description of disease at an early stage, before it is detectable by a physical exam and when it would be more easily treated (*1*).

Molecular events underlie the development of the tissue changes detected in morphologic imaging studies, just as they underlie the histologic changes noted in biopsy specimens. The challenge for diagnostic disciplines is the development of technologies that deliver molecular descriptions of disease to the oncologist. Years of cancer research have uncovered numerous molecules that contribute to an environment conducive to cancer growth and metastasis. A variety of molecules drives the behaviors that are needed for a cancer to form, invade surrounding stroma, and metastasize. To survive a metastatic journey, cells from a primary tumor must detach, migrate within surrounding stroma, cross endothelial cell barriers, survive the intravascular environment, extravasate, continue to migrate, invade the new stroma, form micrometastases, and further proliferate (*2*).

From the perspective of personalized medicine, the molecules that enable this metastatic process to unfold are simultaneously the biomarkers of the disease process and the targets of therapy. Many examples of cancer-relevant molecular interactions have been identified, including those involved in cell–matrix interactions, receptor–growth factor interactions, avoidance of apoptosis, cell motility and associated chemotactic factors, and intracellular signaling pathways that stimulate cell proliferation.

Basic and Translational Research

Immortalized cell lines, primary cultured cells, animal studies, and immunohistochemical studies of patient tissues have provided a rich portrait of cancer pathogenesis. Detailed information has been accumulated at the bench regarding mechanisms whereby a cancer cell senses its environment and thrives within it. A pivotal next step is to determine whether these molecules can be used as in vivo biomarkers of disease.

Direct Study of Human Tissue is Essential

Translational research is focused on understanding how insights gained in the laboratory can be applied to patient disease for diagnostic and therapeutic applications. The role that cell adhesion molecules, such as the integrins, play in mediating intracellular signals highlights the importance

of studying a cancer within the 3-dimensional (3D) context of a tissue specimen. This 3D tissue microenvironment consists of extracellular matrix, capillaries, stromal cells, inflammatory cells, and other cancer cells. All of these molecules and cells coalesce to form a dynamic community (*3*). Extraction of cancer cells from this community deprives them of the environmental cues that characterize their native state. Studies with prostate cancer cell lines and laser capture–microdissected (LCM) prostate cancer tissue demonstrated that the molecular profiles derived from 2 sample sets were markedly distinct (*4*). Moreover, protein microarray studies have indicated that primary and metastatic tumors have divergent protein signaling pathways, suggesting that imaging biomarkers and therapeutic targets will likely be distinct for a primary focus of cancer cells and cells at a site of distant metastatic spread (*5*). Molecules linked to cancer pathogenesis and discovered using actual patient specimens have the highest likelihood of being translated into imaging biomarkers, because they occur within a complex, 3D tumor microenvironment that is impossible to duplicate at present using laboratory methods. Human tissue specimens from patients are therefore becoming a precious resource for understanding the pathophysiology of human cancer.

Molecular Profiling

High-throughput molecular profiling techniques have uncovered an enormous new repository of information about the molecules present within cellular systems. Disease-susceptibility genes, therapeutic targets, and expression profiles linked to disease outcomes have been discovered using genomic microarray assays (*6–8*). Genomic profiling by itself, however, provides an incomplete view of the expressed, functional protein component of a cell's molecular machinery. It has proven difficult to correlate gene transcript levels with protein expression (*9*). In addition, the level and nature of posttranslational modifications as well as protein/protein interactions, which are fundamental to protein cellular activities, are not detected using a solely genomics-based approach (*10,11*). Immunohistochemistry and tissue microarrays are limited in their ability to measure the level of expression of proteins of interest, because the scoring is subjective and poorly quantitative. Measuring the functional protein products within tissue specimens presents challenges for the following reasons: (a) the proteins of interest are of low relative abundance; (b) proteins have no intrinsic signal amplification mechanism as in polymerase chain reaction (PCR); and (c) reagents that specifically detect posttranslationally modified proteins must be generated. To meet these challenges, new proteomic technologies, such as protein microarrays, are being developed and tested (*12,13*).

Current State of Tissue Fixation

To date human tissue processing procedures have relied primarily on fixation processes that prevent or arrest autolysis and putrefaction and preserve the morphology of tissues for pathological evaluation. Buffered formalin has been a mainstay of tissue preservation, enabling further tissue processing and selective uptake of histochemical stains such as hematoxylin and eosin. Differential uptake of these stains by the molecules within tissue specimens provides the distinctive hues of pink to blue that underpin morphologic descriptions to date. This preservation system has proved fairly flexible, as the introduction of antibody reagents for immunohistochemical detection has been integrated into pathological and research practice with the addition of antigen retrieval techniques. Formalin fixation is also compatible with other molecular tissue studies, such as PCR and in situ hybridization. Formalin-based fixation via molecular cross linking, however, interferes with high-throughput assessments of proteins within patient tissues. Alternative preservation methods will be required to achieve uniform results from tissue studies.

An Example of a Molecular Profiling System for Signaling Proteins

To illustrate a current platform for molecular analysis of tissues, reverse phase protein microarrays will be described. A biopsy obtained from a suspicious nodule is snap frozen in embedding medium at –80°C. A frozen section is obtained and stained with a nuclear stain. Tumor cells are microdissected from the tissue section using LCM. At this point the cells obtained using LCM can be processed in different ways depending on the type of molecule of interest (protein, RNA, or DNA). For proteomic studies, the purified tumor cells are lysed under denaturing conditions. The lysate is then arrayed onto a nitrocellulose-coated glass slide in protein spots in a serial dilution curve. Each of the protein spots consists of a heterogenous mixture of analytes. Analytes within the protein spots are probed with antibodies that detect certain isoforms of signaling proteins, such as phosphoproteins. The presence of phosphorylated isoforms can be detected in a spotted lysate representing less than 10 cell equivalents (*14*). Protein microarrays enable a large number of molecular end points to be studied from a single patient biopsy.

Summary

As molecular profiling of patient tissue specimens matures, a subset of molecules linked to disease states and/or response to therapy will provide candidate imaging biomarkers. At present, an important step in the maturation of translational medicine is to move diagnosis from a largely morphologic process to one enhanced by molecular descriptions. As the proteomic contents of cancer cells are cataloged and correlated with disease pathogenesis and response to therapeutic regimens, it is envisioned that lists of molecules that can function as disease classifiers will emerge. These biomarkers are potentially useful as targets for imaging and in pathology evaluations. With these imaging biomarkers, it will be possible to monitor in real time the dysregulation of signaling pathways within the cells of

a tumor smaller than a centimeter and also to pinpoint the sites of disease within the body. Today a patient must wait for a tumor to manifest itself based on its properties as an enlarging physical mass, requiring detection by symptom or physical exam. The goal for the future is to image a subcentimeter focus of tumor and identify therapeutically relevant components of its functioning molecular state in situ before biopsy. This would also open the way for monitoring tumor response to signaling protein therapies. Because metastatic disease is a distinct entity from the primary tumor, molecular imaging would provide insight into signaling pathway dysregulation or other molecular dysfunctions that are present at distinct sites of disease.

Challenges

It is exciting to envision a future in which elements of diagnostic imaging and pathology merge to create a hybrid clinical specialty that delivers personalized diagnostic and therapeutic information about a patient's disease prior to biopsy. Although signaling proteins have been a focus for discussion, molecules that mediate cell–matrix interactions, receptor–growth factor interactions, avoidance of apoptosis, and cell motility are all within the repertoire of potential future disease classifiers.

The critical challenges that stand between current state-of-the-art assessments largely based on morphologic evaluations and this proposed quantum leap in personalized care include the following:

(1) Transition from current tissue collection systems into a molecular profiling–friendly system. Current formalin-centric tissue processing systems are taken for granted but required many years to evolve into the present state. The pressure to build translational medicine programs is forcing a much more rapid timeline for the establishment of uniform tissue collection and processing (of note are the recent special National Cancer Institute requests for applications for funding for tissue collection and processing investigations). Movement toward molecular profiling requires the willingness of interventional imaging physicians, pathologists, oncologists, primary care physicians, surgeons, nurses, and hospital administrators to engage in shared research and testing of tissue collection and preservation systems. The likelihood for success in this shared endeavor is directly tied to the quality of the material that is initially obtained for molecular profiling studies.

(2) Development and application of high-throughput platforms for isolating tumor cells and profiling the molecular content. One example of such a system is an LCM combined with protein microarray technology as described previously. Pathology departments will need to expand the capabilities of their laboratories in order to move into personalized diagnostics.

(3) Multicenter research studies to identify consensus panels of potential molecular imaging biomarkers. Interdisciplinary teams of molecular imaging basic scientists, imaging physicians (nuclear medicine and interventional radiologists), oncologists, pathologists, bioinformaticists, epidemiologists, and other basic scientists will need to engage in collaborative research to profile patient tissue specimens, identify classifying biomarkers, and generate consensus lists of biomarkers relevant to targeted disease entities. Development of information technology systems that fully integrate the many facets of a patient's history and clinical picture with information gained from molecular profiling is required. Molecular medicine will likely cause traditional boundaries between medical disciplines to blur and become less rigid. It will be important for medical specialists to be flexible and willing to adjust to the development of a new molecular medicine paradigm.

(4) Creation of molecular imaging agents that bind molecular targets in their native conformation. Profiling of molecules such as signaling proteins largely depends on detection of denatured analytes using antibodies. Once molecular targets are identified, reagents that interact with nondenatured biomarker analytes within complex tissue settings must be developed and tested.

(5) Development of screening serum biomarkers to identify patients who need molecular imaging studies. The ability to detect and molecularly classify tumors smaller than 1 cm in size 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*). 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 imaging studies.

REFERENCES

1. Etizioni R, Urban N, Ramsey S, et al. The case for early detection. *Nat Rev Cancer.* 2003;3:243–252.
2. Geho DH, Bandle RW, Clair T, Liotta LA. Physiological mechanisms of tumor-cell invasion and migration. *Physiology* (Bethesda). 2005;20:194–200.
3. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. *Nature.* 2001;411:375–379.
4. Ornstein DK, Gillespie JW, Paweletz CP, et al. Proteomic analysis of laser capture microdissected human prostate cancer and in vitro prostate cell lines. *Electrophoresis.* 2000;21:2235–2242.
5. Sheehan KM, Calvert VS, Kay EW, et al. Use of reverse phase protein microarrays and reference standard development for molecular network analysis of metastatic ovarian carcinoma. *Mol Cell Proteomics.* 2005;4:346–355.
6. Baak JP, Path FR, Hermsen MA, Meijer G, Schmidt J, Janssen EA. Genomics and proteomics in cancer. *Eur J Cancer.* 2003;39:1199–1215.
7. Segal E, Friedman N, Kaminski N, Regev A, Koller D. From signatures to models: understanding cancer using microarrays. *Nat Genet.* 2005;37(suppl): S38–S45.
8. Brennan DJ, O'Brien SL, Fagan A, et al. Application of DNA microarray technology in determining breast cancer prognosis and therapeutic response. *Expert Opin Biol Ther.* 2005;5:1069–1083.

9. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol.* 1999;19:1720–1730.
10. Celis JE, Gromov P. Proteomics in translational cancer research: toward an integrated approach. *Cancer Cell.* 2003;3:9–15.
11. Hunter T. Signaling: 2000 and beyond. *Cell.* 2000;100:113–127.
12. Macbeath G. Protein microarrays and proteomics. *Nat Genet.* 2002;32(suppl): 526–532.
13. Liotta LA, Espina V, Mehta AI, et al. Protein microarrays: meeting analytical challenges for clinical applications. *Cancer Cell.* 2003;3:317–325.
14. Paweletz CP, Charboneau L, Bichsel VE, et al. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene.* 2001;20:1981–1989.
15. Deutsch EW, Eng JK, Zhang H, et al. Human Plasma Peptide Atlas. *Proteomics.* 2005;5:3497–3500.

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Recent Trends in Radionuclide-Based Molecular Imaging

Radionuclide-based molecular imaging can utilize nuclides with varying decay properties and half-lives. Although significant interest focuses on the application of SPECT in molecular imaging, the major advances over the past several years have utilized PET. Several factors explain this trend, including:

(1) The availability of a wide variety of radiopharmaceuticals labeled with positron-emitting radionuclides that can be used to study molecular function in a whole variety of diseases. These include: cancer, cardiac disease, pulmonary disease, infection, and inflammation.
(2) The ability to obtain truly quantitative data utilizing PET imaging. Although techniques to quantify SPECT images have improved over the past several years, the ability to obtain truly quantitative data with PET is an advantage.
(3) Modern SPECT instrumentation, both for animal and human studies, has improved in resolution over the past several years. This achievement, however, has been made with a corresponding sacrifice in sensitivity. The higher sensitivity of PET imaging allows greater use of dynamic imaging and the ability to obtain quantitative metabolic and other functional parameters.
(4) The ability to obtain very high specific-activity radionuclides and, hence, radionuclide-based molecular imaging probes allows (in principle) the rapid translation of radionuclide-based molecular imaging from concept to animal studies to patient studies. This has allowed the investigation of such parameters as neuroreceptor imaging, measurements of quantitative cardiac metabolism, tumor metabolism and receptors, as well as other parameters, such as the efficacy of gene therapy. Other techniques under investigation include those to monitor trafficking of specific groups of separated cells.

Issues remain to be addressed in radionuclide-based imaging techniques. Among these are questions related to coregistration. The development of combined PET/CT scanners has allowed major expansion of molecular imaging utilizing 2-fluoro-2-deoxyglucose (^{18}F-FDG) for tumor imaging. The addition of CT to PET, however, produces an increased radiation dose. In animal imaging, where research has been carried out on combined PET/CT scanners and combined SPECT/CT, the radiation dose is significant. The development of a truly integrated MR/PET scanner would be a tremendous advantage.

Although research groups have described radiopharmaceuticals to measure many of the parameters discussed here, more than 90% of human PET studies involve a single tracer: ^{18}F-FDG. New agents face significant barriers in translating to clinical use. These barriers include but are not limited to:

(1) Intellectual property. A significant number of promising radiopharmaceuticals are not patent protected. It is unlikely that a commercial company will invest significant funds to translate these agents to the clinic.
(2) Although many regional radiopharmacies now produce ^{18}F-FDG, many of the other positron-emitting radiopharmaceuticals under investigation have low radiochemical yield. This makes the commercial applicability of these somewhat limited. With the current funding levels at the National Institutes of Health, it is unlikely that funding can be obtained to simply increase the radiochemical yield of a validated radiopharmaceutical.
(3) Although the requirements for Investigational New Drug Applications by the U.S. Food and Drug Administration have been simplified, human use approval of new radioactive probes is still perceived as a roadblock.

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