Meeting Report

Special Conference of the American Association for Cancer Research on Molecular Imaging in Cancer: Linking Biology, Function, and Clinical Applications in Vivo

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Abstract

The AACR Special Conference on Molecular Imaging in Cancer: Linking Biology, Function, and Clinical Applications In Vivo, was held January 23–27, 2002, at the Contemporary Hotel, Walt Disney World, Orlando, FL. Co-Chairs David Piwnica-Worms, Patricia Price and Thomas Meade brought together researchers with diverse expertise in molecular biology, gene therapy, chemistry, engineering, pharmacology, and imaging to accelerate progress in developing and applying technologies for imaging specific cellular and molecular signals in living animals and humans. This format of the conference was the presentation of research that focused on basic and translational biology of cancer and current state-of-the-art techniques for molecular imaging in animal models and humans. This report summarizes the special conference on molecular imaging, highlighting the interfaces of molecular biology with animal models, instrumentation, chemistry, and pharmacology that are essential to convert the dreams and promise of molecular imaging into improved understanding, diagnosis, and management of cancer.

Conference Summary

Donald Coffey (Johns Hopkins, Baltimore, MD) delivered the meeting keynote address, which outlined the challenges facing investigators in the field of molecular imaging. During tumor initiation, progression, and metastases, cancer cells demonstrate marked heterogeneity because of genetic instability. Investigating causes and outcomes of this genetic heterogeneity is one potential application of molecular imaging for studying cancer in animal models and improving diagnosis and treatment of patients with cancer. As discussed by Dr. Coffey, a key to acceptance of molecular imaging technologies for research and therapy will be the identification of unique molecular signatures for cancer cells and the rigorous validation of imaging techniques as informative tools for detecting these markers.

In general, speakers presented two alternative approaches for developing technologies and reagents for molecular imaging. The first strategy uses de novo synthesis of unique imaging agents targeted to a specific molecular marker, such as a receptor or transporter. Using this method, iterative modifications in the structure of compounds are made to develop an agent for imaging a target of interest. For each novel target, the sensitivity and specificity of interaction, pharmacokinetics of delivery, and signal-to-noise for an imaging agent must be characterized by appropriate in vitro and in vivo assays. Because of the time and effort involved in developing and validating a specific imaging agent, researchers emphasized the need to select carefully an important molecular marker for cancer initiation, progression, or response to therapy.

Reporter proteins are the second general strategy used by researchers to image specific molecular and cellular events. Initial development of a reporter system is as described for the de novo synthesis paradigm, involving selection and biochemical characterization of a reporter protein and imaging agent to interrogate the reporter in vivo. However, the final result of this approach is to engineer a general system that may be used to image many different biological processes. Reporter proteins are an indirect method to visualize transcriptional and posttranscriptional regulation of gene expression, protein-protein interactions, or trafficking of proteins or cells in vivo. Thus, investigators described the importance of determining how accurately the reporter protein reproduces regulation and function of the corresponding endogenous pathway, thus proving that the reporter does not perturb the underlying biology. Because the reporter protein paradigm necessitates introducing one or more foreign proteins into a cell, delivery of the reporter gene may be a limitation of this strategy in intact animals, particularly humans. For this reason, several researchers proposed that reporter proteins might be more difficult to translate to clinical applications than de novo synthesis of imaging agents.

De Novo Synthesis Paradigm

William Eckelman (NIH, Bethesda, MD) described the significance of exploiting data from genomics and proteomics research to identify new targets for imaging. To prove selectivity of a new imaging agent for a target, he summarized essential principles for pharmacological validation of imaging probes in vivo. Interaction with the target should be saturable and specific, as evidenced by displacement with competing compounds and correct anatomical localization both macroscopically and microscopically. The imaging agent should show sterosepecificity, and differences in accumulation in vivo should correlate with relative differences in affinity. As shown by studies with the M2 muscarinic receptor, genetically engineered mouse models can provide important data for validating specificity of an imaging probe, limited by the caveat that mice are not guaranteed to faithfully reproduce human physiology. These basic principles of development and characterization of targeted agents for molecular imaging were emphasized in several presentations.

Several speakers focused on development of peptide agents for molecular imaging of markers in cancer. Vascular endothelia in normal and malignant tissues contain unique ligands ("molecular zip codes") that can be detected in mouse models and human patients. Renata Pasqualini (University of Texas, M. D. Anderson Cancer Center, Houston, TX) described the human vascular mapping project, which uses phage display libraries to screen for peptides that bind to specific vascular endothelia in vivo. Using sequence data from the human genome project, she has identified a large number of proteins that confer selective targeting of peptides to defined anatomical sites. These data can be used to identify new targets for molecular imaging, as discussed by Markus Schwaiger (Nuklear Medizinische Klinik,
Munich, Germany). Peptides containing an RGD amino acid motif bind with high specificity to α5β1 integrins expressed during angiogenesis. Radiolabeled, cyclic pentapeptides with RGD amino acids bind specifically to α5β1 integrins in the neovasculature of animal tumor models, and clinical trials of this imaging agent are scheduled to begin soon.

Silvia Jurisson (University of Missouri, Columbia, MO) presented work with novel cyclized analogues of α-MSH\(^4\) targeted to the α-MSH receptor. Because expression of this receptor is increased in melanoma, Tc- and Re-labeled peptides potentially could be used for molecular imaging and radiotherapy, respectively, in patients with melanoma. Claude Meares (University of California, Davis, CA) discussed strategies to improve \(in vivo\) targeting and detection of antibodies for molecular imaging. By engineering the system to form a covalent bond between ligand and antibody, he prevented dissociation of the ligand-antibody pair, resulting in enhanced target-to-background signal from his radiolabeled imaging agent. Similar to the α-MSH peptides, the antibody system described by Dr. Meares also could be used for targeted radiotherapy \(in vivo\).

Other presentations focused on customized chemical synthesis of organic and inorganic compounds to optimize specific interactions with a molecular target. John Katzenellenbogen (University of Illinois, Urbana, IL) discussed structural studies of the ER that enabled him to design new estrogen analogues that bind to various ER mutants. His data showed that ER mutants and estrogen analogues could be designed to maintain high binding affinity of the matched ligand-receptor pair while significantly reducing cross-reactivity with endogenous ligands and receptors. Unique pairs of mutant ER and estrogen analogue could be used as unique reporter genes for \(in vivo\) imaging with minimal or no cross-reactivity with other receptors. Hank Kung (University of Pennsylvania, Philadelphia, PA) presented radiochemistry and biochemistry of an imaging agent for sigma-2 receptors, which may serve as molecular markers of proliferation in cancers such as breast, lung, and prostate, and melanoma. To optimize pharmacokinetics and \(in vivo\) selectivity for sigma-2 receptors compared with the related sigma-1 receptor, high-performance liquid chromatography isolation of isomers from the starting racemic mixture of radio-tracer was necessary. Thomas Meade (California Institute of Technology, Pasadena, CA) described synthesis and targeting strategies for biochemically activated contrast agents for MRI. Unlike steroid analogues or imaging agents directed at cell surface receptors or transporters, Dr. Meade's molecular targets were intracellular enzymes, such as the reporter enzyme β-galactosidase. To direct intracellular delivery of MRI imaging agents, he synthesized and biochemically characterized a number of small molecule chaperones that facilitate the transport of contrast agents across cell membranes without preventing activation of the agent. The use of these imaging molecules may enable combined anatomical and functional imaging of cell fate and differentiation with high-resolution MRI.

Synthesis of targeted imaging agents also included development of nucleic acid probes, as discussed by Bertrand Tavitian (CEA/SHFJ/INSERM MO103, Orsay, France) and David Ward (Yale University School of Medicine, New Haven, CT). Dr. Tavitian analyzed stability and biodistribution of modified oligonucleotides as antisense imaging agents. Modifications that improved \(in vivo\) stability of labeled RNA molecules showed only nonspecific binding to tissues. Conventional phosphodiester RNA molecules, which are degraded rapidly in serum, showed enhanced target-to-background signal, but intracellular trans-}

\(^4\) α-MSH, α-melanotropin-stimulating hormone; ER, estrogen receptor; MRI, magnetic resonance imaging; GFP, green fluorescent protein; PET, positron emission tomography; HSV1-tk, herpes simplex virus-1 thymidine kinase; Pgp, P-glycoprotein; FDG, \({}^{18}\)fluorodeoxyglucose.

**Reporter Protein Paradigm**

GFP and related fluorescent proteins are commonly used reporters for molecular biology studies because they can be visualized directly in living cells without fixation or substrate delivery. These reporters typically are used as fluorescent molecular labels to localize proteins within cells. However, several presentations addressed innovative applications fluorescent reporters in cultured cells and model organisms. Robert Singer (Albert Einstein, Bronx, NY) discussed GFP as a reporter for tracking movements of mRNA in cells. Using real-time imaging of chimeric-GFP reporters, he demonstrated that untranslated regions within the mRNA molecule itself regulate asymmetric distribution in polarized cells. Localization of mRNA within cells provides a mechanism to control protein sorting and expression, protein-protein interactions, and asymmetric distribution of proteins during development. Martin Chalfie (Columbia University, New York, NY) used GFP to analyze development and differentiation of touch receptor neurons in \(Caenorhabditis elegans\). GFP reporters marked specific cell populations, thus enabling characterization of worms with mutant phenotypes. GFP labeling also identified specific neurons, providing a means to perform electrophysiology on single cells or to select populations of touch receptor cells by flow cytometry for microarray analysis. Roger Tsien (University of California, San Diego, La Jolla, CA) discussed genetically encoded fluorescent proteins for imaging posttranslational modifications of proteins, such as kinase and phosphatase activities, and for detecting intracellular concentrations of calcium in living cells. He also described development and application of FIAAS (fluorescein-based arsenical hairpin binder) for fluorescent labeling of recombinant proteins in intact cells. Imaging of GFP in intact animals has been reported previously. However, translation of these advanced applications of fluorescent reporters from cultured cells to living animals will require significant advances in imaging technologies and instrumentation, and Dr. Tsien suggested that non-optical approaches may be needed to image posttranslational protein modifications in \(in vivo\).

Firefly (Photinus pyralis) luciferase is another reporter protein that is commonly used for studies in cultured cells. Using a sensitive detection system based on a cryogenically cooled charge-coupled device camera, optical imaging of luciferase is feasible in living mice, thus allowing direct translation of this reporter system from \(in vitro\) to \(in vivo\) applications. Christopher Contag (Stanford University, Stanford, CA) discussed \(ex vivo\) transduction of tumor cells with a luciferase reporter for spatial and temporal studies of tumor cell trafficking in mouse models. To allow selection and characterization of cell populations, he engineered reporter constructs to coexpress luciferase and a fluorescent protein. He also presented data showing that responses of tumors to chemotherapy and/or immunotherapy could be detected noninvasively \(in vivo\), based on changes in relative amounts of transmitted light from luciferase-expressing cancer cells. Brian Ross (University of Michigan, Ann Arbor, MI) presented data showing that luciferase can be used as a reporter for molecular events of
apoptosis in vivo. Luciferase activity was silenced by mutant ERs that were linked to the reporter by a peptide sequence that included the cleavage motif for caspase 3. Activation of caspase 3 during apoptosis removed the ERs, resulting in enhanced bioluminescence. He constructed similar reporters for imaging caspase 8 and 9, and the same strategy should be applicable to molecular imaging of other intracellular proteases.

Other investigators presented applications of reporter proteins designed for PET, an imaging modality that can be used for both animal models and humans. Ronald Blasberg (Memorial-Sloan Kettering Cancer Center, New York, NY), used a fusion protein of wild-type HSV1-tk and GFP as a reporter for PET imaging of transcriptional activity in pathways regulated by p53, hypoxia, transforming growth factor β, and NFAT (nuclear factor of activated T cells). Potential applications of these reporter technologies included studying specific signaling pathways in transgenic mice, quantifying responses to drugs targeted to a pathway of interest, and monitoring targeting and persistence of vectors in gene therapy. Drs. Blasberg and David Piwnica-Worms (Washington University School of Medicine, St. Louis, MO) also described research about enzymatic activity of HSV1-tk or mutants of this reporter protein for various radionucleotide substrates. Their presentations emphasized the need to validate the biochemistry of pairs of reporter protein and imaging substrate to understand and optimize systems for molecular imaging. Dr. Piwnica-Worms presented one example in which matching of a mutant HSV1-tk with an imaging agent provided the sensitivity necessary to detect protein-protein interactions in living mice with a PET imaging system designed for research on small animals (microPET).

Harvey Hershman (University of California-Los Angeles School of Medicine, Los Angeles, CA) discussed specific applications of PET imaging for noninvasive, quantitative monitoring of gene therapy in vivo. Using an internal ribosomal entry site (IRES) to coexpress a defunctionalized dopamine 2 receptor (D2R) with a mutant HSV1-tk in living mice, he showed that both PET reporter proteins could be detected and quantified by microPET imaging. Furthermore, activities of both reporters were correlated highly over time, demonstrating the feasibility of using a PET reporter to monitor spatial localization and relative expression of a therapeutic transgene. The potential for direct translation of this imaging paradigm to cancer gene therapy with viral vectors was highlighted by William Wold (St. Louis University, St. Louis, MO). Dr. Wold described adenoviral vectors engineered to conditionally replicate in cancer cells and produce pro-apoptotic proteins such as tumor necrosis factor-related apoptosis-inducing ligand, resulting in selective killing of tumors in animal models. He currently is incorporating HSV1-tk into these oncolytic adenoviruses to enable PET imaging of viral distribution and persistence.

James Basilion (Massachusetts General Hospital, Charlestown, MA) described the transferrin receptor as a reporter protein for MRI, using transferrin-iron oxide particles as an imaging agent. Although MRI offers superior spatial resolution compared with other external imaging technologies (luciferase or PET), MRI is significantly less sensitive for detecting the accumulation of an imaging agent. Thus, both the biology of the receptor system and chemistry of the imaging agent had to be optimized to amplify the signal for successful molecular imaging. Dr. Basilion also described initial work with microarray analyses of dissected tumor samples with the ultimate goal of identifying multiple markers in tumors that could be imaged simultaneously for diagnosis and prognosis in patients with cancer.

Development of New Instrumentation for in Vivo Imaging

To enable molecular imaging of small animals, engineers and physicists have developed technologies for bioluminescence imaging (IVIS, Xenogen Corporation) and microPET. These instruments provide the sensitivity and spatial resolution necessary for imaging luciferase and various PET reporter proteins, respectively. In addition, two presentations focused on design of novel equipment for defining surface anatomy of tissues with optical techniques, using probes that are small enough to image humans with minimally invasive techniques such as endoscopy. Calum MacAuley (British Columbia Cancer Agency, Vancouver, BC, Canada) described instrumentation and image processing techniques for performing confocal microscopy in patients. Size, shape, and spatial arrangement of cell nuclei were detected and quantified with this method, enabling differentiation of normal and dysplastic epithelial surfaces. This imaging modality may allow early detection of epithelial cancers in patients and allow investigators to monitor effects of chemoprevention. James Fujimoto (Massachusetts Institute of Technology, Cambridge, MA) discussed applications of optical coherence tomography (OCT), a technique that uses reflected light to define surface anatomy of tissues with resolution as low as 1 μm. This instrument is in clinical practice for identifying structural changes in the retina, and “optical biopsies” with OCT also may be effective for detecting distortion of tissue anatomy in early cancers.

Translating Molecular Imaging to Clinical Practice

Many molecular imaging technologies have been developed and characterized in cultured cells and animal models. Although studies in mice likely will significantly advance research into cancer initiation and treatment, several speakers emphasized the need to translate molecular imaging technologies to clinical management of patients. Paul Workman (Institute of Cancer Research, Surrey, United Kingdom) described the importance of determining pharmacokinetics and pharmacodynamics of new drugs early during clinical development. Potentially, molecular imaging could be used to replace the invasive techniques that currently are used to determine delivery of drug to tumor tissue and the effect on a specific molecular target. Dr. Workman also stated that developing imaging agents to monitor downstream steps in signal transduction pathways might make the assays useful for more than one drug and target. Patricia Price (Christie Hospital, Manchester, United Kingdom) provided examples of how PET imaging is currently being used in clinical trials of chemotherapeutic drugs to address relevant questions of pharmacokinetics and pharmacodynamics that were outlined by Dr. Workman. Merrill Egorin (University of Pittsburgh Cancer Institute, Pittsburgh, PA) also emphasized the potential for molecular imaging to measure the accumulation of chemotherapeutic drugs in tumors and assess modulation of the desired target. However, he also cautioned that the value of molecular targeting of drugs and molecular imaging of treatment effects must ultimately be determined by improved survival of patients with cancer.

Three presentations described molecular imaging technologies that are currently used in clinical trials and/or patient management. MDR1 Pgp is an ATP-binding cassette transporter that is expressed normally in various tissues and is overexpressed during initiation or progression of some cancers. Michael Gottesman (National Cancer Institute, Bethesda, MD) detailed the biochemistry of this and related ABC transporters that confer resistance, to structurally diverse chemotherapeutic drugs. Building on the basic science of MDR1 Pgp function, James Ballinger (Addenbrooke’s Hospital, Cambridge, United Kingdom) discussed radiotracers, such as Tc99m-Sestamibi, that are molecular imaging probes for the transporter. Clinical trials are in progress to determine whether nuclear medicine imaging of MDR1 Pgp can be used to detect inhibition of the transporter by specific modulators or provide diagnostic information for cancer patients.
Richard Wahl (Johns Hopkins University, Baltimore, MD) discussed applications and diagnostic significance of functional PET imaging with FDG, an imaging examination that currently is used clinically in patients with cancer. Because glucose metabolism is increased in many types of cancer through mechanisms such as up-regulation of GLUT1 transporters, accumulation of FDG is established as a sensitive and relatively specific imaging assay for detection of many primary and metastatic tumors. Changes in FDG accumulation have been shown to predict treatment response for some malignancies. Dr. Wahl also described the improved diagnostic accuracy obtained by imaging patients with newly developed hybrid computed tomography-PET scanners that allow fusion of anatomical and functional data. Molecular imaging of somatostatin receptor 2 (SSTR2) with radiolabeled octreotide or octreotate (analogues of somatostatin) also is used in clinical management of patients with neuroendocrine tumors, as discussed by Roelf Valkema (Erasmus University Medical Center, Rotterdam, the Netherlands). Planar and tomographic imaging with In\textsuperscript{111}-labeled somatostatin analogues is more sensitive for detection and staging of neuroendocrine tumors than is anatomical imaging with MRI or computed tomography. Similar to proposed applications of other targeted imaging agents, clinical trials are in progress to determine whether radiolabeled octreotate can be used for therapy in tumors that express SSTR2.

Conclusions

In summary, presentations by invited speakers and data from poster sessions highlighted the rapid progress that has been made in the new field of molecular imaging. Molecular imaging biotechnologies have been applied successfully \textit{in vivo} to studies of transgene delivery, tumor cell trafficking, drug efficacy, and detection of selected molecular profiles of tumors. By developing and validating new imaging technologies to interface with data obtained from genomics and proteomics, molecular imaging has the potential to further advance cancer research in animal models and clinical practice. As evidenced by the diverse scientific backgrounds of participants at the meeting, interdisciplinary collaborations have been crucial to the rapid advances in molecular imaging and will continue to be essential for further progress. However, participants at the conference also departed with the knowledge that both innovation and careful validation of biotechnologies will be necessary to fully integrate molecular and cellular biology with \textit{in vivo} imaging.
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