Journal of Cellular Biochemistry Supplement 39:90–97 (2002)

Molecular Imaging, Targeted Therapeutics, and Nanoscience

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Abstract The recent emergence of ''molecular imaging'' as an academic discipline has set the stage for an evolutionary leap in diagnostic imaging. Recent advances in nuclear, ultrasound, optical, and magnetic resonance imaging have generated interest in molecular imaging across all modalities and across various academic, industrial, and governmental agencies. In this perspective, examples of the progress and the prospects for the future of molecular imaging and linked targeted therapeutics are reviewed. J. Cell. Biochem. Suppl. 39: 90–97, 2002. © 2002 Wiley-Liss, Inc.

Key words: molecular imaging; nanotechnology; magnetic resonance imaging

Since the inception of diagnostic radiography following Roentgen's discovery of X-rays in 1895, the art of noninvasive diagnostic imaging largely has dealt with cataloguing of morphologic descriptors of disease processes based on pattern recognition or feature extraction. Diagnoses have relied primarily on registering and interpreting intrinsic differences in image contrast between normal and abnormal tissues. A great deal of clinical experience is required to juxtapose and compare the findings in a single case with the recorded patterns that imply disease processes, all of which must be recalled from memory.

Additional challenges exist. Image contrast between normal and abnormal tissues is modest in most instances, and must be factored with the actual geometry of the lesion to gauge tissue characteristics. Assessment of lesion geometry depends on image resolution, which typically is a few hundred microns at best, and which determines the smallest size, or earliest stage, of a pathology that can be diagnosed. And, image noise distorts the signals, leading to inaccuracy and/or uncertainty of diagnoses. Image formation is further complicated by patient-dependent motion artifacts, and by a host of technological factors that include the performance of the imaging equipment and the experience of the operator. Under the present paradigm for nondestructive image-based diagnosis, one may be left with visualizing a "shadow," or a "reflection," or a "hot" or "cold" spot of undetermined cause and significance, which may require invasive workup.

The recent emergence of "molecular imaging" as an academic discipline has set the stage for an evolutionary leap in diagnostic imaging [Allport and Weissleder, 2001]. "Molecular imaging" is not a substitute for the traditional process of image formation and interpretation, but is meant to improve diagnostic accuracy by providing an in vivo analog of immunocytochemistry or in situ hybridization. It is less concerned with native image contrast or resolution, which are keys for depicting the effects of the disease on surrounding normal tissues, but rather seeks to enhance the conspicuity of subtle pathologies by targeting the molecular components or processes that are causes of disease. Previous advances in nuclear imaging, together with more recent efforts in ultrasound, optical, and MRI have energized interest in molecular imaging across all modalities and across various academic, industrial, and governmental agencies. In this perspective, we will review the process, the progress, the players, and the prospects for

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Received 11 October 2002; Accepted 15 October 2002
DOI 10.1002/jcb.10422
Published online in Wiley InterScience (www.interscience.wiley.com).

the future of molecular imaging and targeted therapeutics, and examine the related opportunities for development of novel biocompatible nanotechnologies as molecular imaging agents.

GENERAL CONCEPTS

The flowering of interest in molecular imaging is exemplified in summary statements from the second Biomedical Imaging Symposium: Visualizing the Future of Biology and Medicine ("http://www.becon1.nih.gov/symposium1999.htm"), which was convened at the National Institutes for Health June 25–26, 1999 and was cosponsored by the Biomedical Engineering Consortium (BECON), the Radiological Society of North America, and the American Institute of Medical and Biological Engineering to: (1) "identify the most important challenges and opportunities in biomedical imaging science" and (2) "develop strategies for integrating imaging science with biological and medical research." The BECON symposium identified five action items critical to achieving these goals that are paraphrased in Table I.

Because molecular constituents of pathological processes are too small to be resolved by clinically applicable imaging modalities, specific and sensitive site-targeted contrast agents typically are employed as beacons for molecules of interest. Unlike a blood pool agent, a site-targeted contrast agent is intended to enhance a specific pathological tissue that otherwise might be difficult to distinguish from surrounding normal tissue. The desired properties of such agents are: long circulating half-life (hours), selective binding to epitopes of interest, low background signal and prominent contrast-to-noise enhancement, acceptable toxicity profile, ease of production and clinical use, applicability with standard commercially available imaging modalities, and promise for adjunctive therapeutic delivery. Clinical availability of these agents could redefine the practice of imaging by delineating cellular and molecular mechanisms of disease, and simultaneously creating an opportunity for adjunctive drug/gene delivery.

Traditional imaging devices can be optimized for these purposes to provide spatial registration and local quantification of selected molecular epitopes. The contrast agents themselves may be actively or passively sequestered with the targets, but they should accumulate with significant specificity and signal strength. The signals produced should be sufficient to provide diagnostic contrast-to-noise ratios at low enough concentrations to limit local and systemic toxicity. Binding to molecular epitopes should be rapid and persistent to enable robust and convenient clinical imaging.

The process of molecular imaging starts with the selection of a molecular target and the development of an agent to bind to it (Fig. 1). Key factors include the abundance and microscopic location of the epitopes. Vascular, interstitial, membrane, intracellular, and nuclear epitopes all may require tailored approaches.

These factors also will influence the choice of imaging modality. Nuclear or optical contrast agents perform well under optimal imaging conditions with simple ligand-radionuclide and ligand-fluorophore constructs that typically require nano- or picomolar concentrations to achieve acceptable contrast-to-noise ratios. The possibilities for ultrasound and MRI agents are more restricted because of the reduced sensitivity that can require a larger carrier construct such as microbubble contrast agents for ultrasound, and paramagnetic nanoparticle-ligand complexes for MRI. Such larger agents may not penetrate directly to all biological sites of interest. However, the advantages of ultrasound or MRI approaches relate to their intrinsically superior resolution as compared with nuclear, and a superior depth of penetration as compared with optical methods.

The contrast mechanism depends on the choice of imaging modality, which itself is determined by the clinical problem and accessibility for imaging. For example, carrier moieties

TABLE I. BECON Action Items

- Implementation of multidisciplinary research programs, especially in the area of molecular imaging or image guided therapy
- Development of new imaging technologies, probes, and contrast agents that are the tools for linking imaging to specific biological processes
- Initiation of new training programs in molecular imaging to create a generation of scientists for whom the principles of imaging, physics, bioengineering, molecular biology, physiology, pharmacology, and pathophysiology form an intellectual continuum
- Planning for new clinical trials combined with informatics approaches to assess biomedical imaging technologies
- Enhancement of cooperation among NIH, FDA, HCFA, and industry (both large and small businesses) to improve the speed with which new imaging technologies, probes, and contrast agents can be transferred into clinical practice

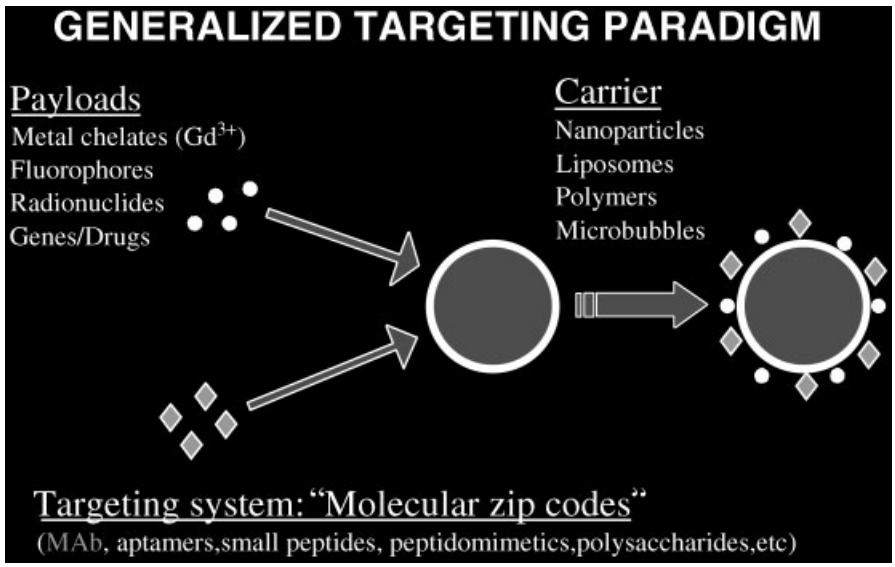

Fig. 1. A paradigm for nanoparticle imaging agents.

such as nanoparticles (liposomes or emulsions) [Lanza et al., 1992, 1996a, 1996b; Sipkins et al., 1998], dendrimers [Wiener et al., 1994; Bryant et al., 1999], viral constructs [Bulte et al., 2001a], or various polymers [Ma et al., 2001; Roessler et al., 2001] can be loaded with large payloads (Fig. 1) of imaging agents such as paramagnetic or supraparamagnetic metals, fluorophores, or radionuclides to enable detection with standard imaging equipment. In the case of ultrasound imaging, the intrinsic physical properties of the carrier agents themselves (density and compressibility) establish the means for detection. For certain constructs, such as liquid perfluorocarbon nanoparticles, [Lanza et al., 1996a, 2000a; Yu et al., 2000] considerable flexibility exists to utilize any or all imaging modalities.

The contrast agent should manifest high affinity and avidity for the target. Generally, the targeting ligands are coupled directly to the carriers and comprise antibodies or their fragments, peptides, small molecule peptidomimetics, or aptamers, which confer specificity of binding (Fig. 1). The rapid expansion of the monoclonal antibody industry has prepared the stage for broad application of site targeted contrast agents by providing a plethora of specific and sensitive ligands that can be directed against a host of molecular epitopes. Phage display technologies and combinatorial chemistry approaches also promise identification of additional ligands. Dissociation constants in the nanomolar range or better are preferred. Multivalent binding can be useful to enhance avidity and reduce "off-rates" so that binding persists long enough to permit imaging at convenient times after delivery of the agent. Polyvalent binding is possible with the use of more than one ligand type per carrier, or with mixtures of ligand-carrier constructs directed at different targets.

Finally, the ability to incorporate drugs or genes into these carriers represents a new paradigm in therapeutics that could usher in an era of image-based drug dosing (Fig. 2). Payloads of therapeutic agents such as genes or radionuclides can be complexed to the carriers themselves. Drugs can be linked to or dissolved within carrier lipid coatings, or deposited in subsurface oil layers, or trapped within the carriers themselves. High drug concentrations at tissue sites are achieved through progressive accumulation of the carrier agents, implying that serum levels can be minimized to reduce harmful side effects. Drug delivery from carriers to cells can occur by diffusion, particle fusion and internalization into cells, component (lipid–lipid) exchange and convective flux, or some combination of these mechanisms [Lanza et al., 2002]. In the case of ultrasound, methods for gene delivery with synthetic vectors such as microbubbles rely on mechanical stimulation of microbubbles that operate like miniature gene guns [Song et al., 2002].

The opportunity to confirm drug delivery and dose by imaging represents a novel feature for agents developed for controlled drug release.

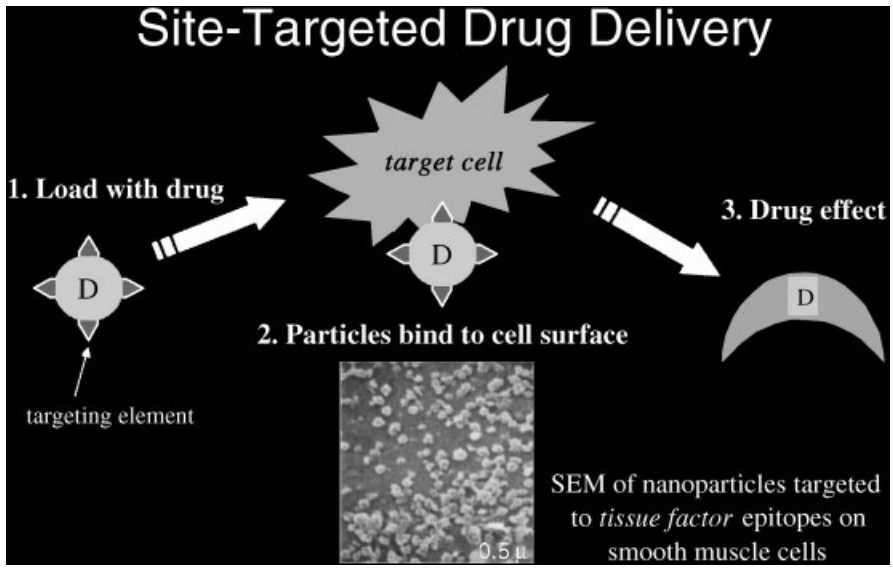

Fig. 2. Delivery of site-targeted therapeutic agents with high payload nanoparticles.

Quantification of systemic levels of such agents would be inutile for ensuring therapeutic drug levels at the tissue since the gradients for drug penetration are now reversed (i.e., from cell to serum). Fortunately, the imaging signals produced by the carriers themselves, along with knowledge of the amount of the specific drug per particle, allow one to estimate local drug concentrations. "Counts per minute" for nuclear imaging, fluorescence intensity for optical imaging, and spectroscopic characterization of carrier signals for MRI all might bear monotonic relationships to carrier/drug concentrations. This possibility ultimately might allow rational drug dosing based on quantification of the local concentrations of the agent, and eventually permit more exquisite titration of especially potent drugs that otherwise would exhibit unacceptable toxicity if employed at high serum levels.

PROGRESS

This section illustrates a few highly selected examples of work in oncologic and cardiovascular molecular imaging. For MRI applications, the candidate agents comprise paramagnetic (e.g., gadolinium-coupled) or supraparamagnetic (e.g., iron oxide bearing) metals linked to various carriers such as liposomes, emulsions, dendrimers, cross-linked copolymer particles, linear polymers, viral capsid particles, and micelles, among others. For example, paramagnetic polymerized liposomes bearing antibody ligands to neovascular integrins $\alpha_v\beta_3$ have been used for targeting experimental tumor angiogenesis, and accumulation after 24 h allows sufficient signal for imaging [Sipkins et al., 1998].

Similarly, liquid perfluorocarbon nanoparticle emulsions targeted to $\alpha_v\beta_3$ also permit robust tumor imaging after only 1 h in the circulation, with specific uptake demonstrated by in vivo competition experiments [Anderson et al., 2000]. Indeed, liquid perfluorocarbon nanoparticles were the first example of molecular targeting agents useful for MRI of thrombi by incorporating antibody ligands directed against cross-linked fibrin [Lanza et al., 1996b], and have been shown recently to be useful for characterizing experimental thrombi in vivo and human unstable carotid plaques ex vivo [Flacke et al., 2001].

These agents also are useful for delivery drugs after binding to cellular epitopes by a mechanism called "contact facilitated drug delivery" (Fig. 2). This results from enhanced lipid–lipid exchange with the lipid membranes of targeted cells, which permits convective flux of drugs dissolved in the outer lipid membrane into the targeted cells [Lanza et al., 2002]. Such agents serve as a depot drug delivery system with prolonged release and long persistence at the site.

For paramagnetic agents, it is important that the longitudinal relaxivity per molecular binding site for the complex is maximized, allowing contrast enhancement with very small numbers

of paramagnetic particles. For paramagnetic perflurocarbon nanoparticles, particle based relaxivities (or unit signal strength) are the highest reported to date in the literature, exceeding that of clinically available MRI paramagnetic contrast agents by at least 10,000X or more [Flacke et al., 2001]. We have estimated that such agents will provide sufficient signal to be detectable at local concentrations in the picomolar range, which heretofore had been thought possible only with nuclear agents. Even single cells can be imaged with such agents [Lanza et al., 2002]. However, for epitopes in high concentrations such as fibrin in thrombi, other novel paramagnetic agents with more modest enhancements in relaxivities ($\sim$1.5X greater than the current agents) can be useful for targeting, as has been reported more recently for targeting fibrin clots in vivo with the use of small peptide ligands [Caravan et al., 2002].

"Susceptibility" or "cold spot" agents have been produced by combinations of carriers with iron oxides (e.g., ultrasmall particles of iron oxide, or USPIO's) or alternative lanthanide species [Bogdanov et al., 1999; Josephson et al., 1999]. Recent work has demonstrated potential for delineation of early atherosclerosis in experimental models by uptake of USPIO's in plaque macrophages [Ruehm et al., 2001]. Stem cell labeling with magnetodendrimers permits MRI detection and precise localization after therapeutic injection [Bulte et al., 2001b]. However, because these agents produce a signal void rather than a hot spot, the susceptibility agents could yield less favorable results for detection of small targets of low prevalence.

Some versions of polymers might be utilized as carriers of magnetic and therapeutic materials as well [Ma et al., 2001; Roessler et al., 2001]. Various transport ligands such as the retroviral "tat" protein have been coupled to these agents to promote cell uptake (e.g., CLIO's) [Kang et al., 2002; Wunderbaldinger et al., 2002]. Most have been prepared as susceptibility ("cold spot") agents, which may suffer the problems outlined above. Those that are paramagnetic may experience the problem of low gadolinium payload, which could limit their usefulness in "hot spot" molecular imaging.

Other so-called "smart" agents are designed to take up residence in all cells of the body but to be activated only by specific enzymes that are expressed in the cell under certain pathologic states, or by the protein products (enzymes) of reporter genes following therapeutic transfection [Louie et al., 2000]. Cleavage of active sites on these agents exposes sequestered gadolinium atoms to free water and facilitates rapid water exchange to produce an effect on local proton relaxation. Despite being highly selective, the sensitivity of such intracellular gadolinium agents with respect to fast free water exchange required to effect proton relaxation has not been reported. Furthermore, robust methods for loading cells after systemic dosing have not been developed.

Agents that take advantage of other modalities include nuclear constructs that are useful for monitoring transfection events by imaging proteins that are expressed after reporter gene transcription. For example, HERPES virus thymidine kinase genes can be used as a reporter construct in association with a therapeutic gene by phosphorylating certain exogenously supplied radiolabeled probes (or substrates) that then are trapped inside of cells where they can be imaged [Bengel et al., 2000; Gambhir et al., 2000]. Radionuclide imaging of cellular apoptosis has been reported with the use of technicium-labeled annexin, which targets membrane phosphatidyl serine epitopes that are exposed during apoptosis [Blankenberg et al., 1998].

The field of optical imaging has undergone explosive growth [Allport and Weissleder, 2001]. This modality exhibits great flexibility in the choice of agents and the detection schemes for multispectral analysis. Confirmation of gene transfection has also been described with based on optical bioluminescence imaging of reporter gene products such as luciferase after supplying the substrate, luciferin [Wu et al., 2002]. Although the method is limited by the need for sensor proximity and it's short depth of penetration and nontomographic image data, for many clinical applications it should assume a prominent role in both diagnostics and adjunctive therapeutics.

For ultrasound, stabilized gaseous microbubble contrast agents ($\sim$5 μm in diameter) also have demonstrated potential for use as transfection agents by incorporating DNA directly into the bubble shell or interior [Unger et al., 1997; Shohet et al., 2000]. The technique involves cavitational destruction of the bubbles with focused ultrasound that is applied externally to release genes at selected sites. The use

of microbubbles as imaging agents generally is restricted to the vasculature in view of their size and susceptibility to destruction with clinical ultrasound imaging intensities, but targeting to thrombi has been reported [Takeuchi et al., 1999].

Targeted perfluorocarbon nanoparticles were the first reported molecular imaging agent for ultrasound applications and were shown to augment reflectivity from fibrin thrombi in vivo by 2 orders of magnitude or more [Lanza et al., 1996a, 1997, 1998]. Additionally, targeting to vascular epitopes such as tissue factor, whose expression is induced in smooth muscle cells in vivo after angioplasty, is possible because these particles can penetrate through microfissures into the vascular media [Lanza et al., 2000a, 2000b]. Reflective liposomes also have been used to specifically target endothelial integrins [Lanza et al., 1992; Demos et al., 1999].

THE PLAYERS

The growth of molecular imaging has been nurtured primarily by the academic community. The Academy of Molecular Imaging (http://www.ami.org) was formed as a consortium of clinical researchers initially devoted to nuclear and PET imaging. Recent efforts of this group have helped persuade the FDA to approve the use of PET for clinical evaluation of breast cancer. The Society of Molecular Imaging (http://www.societymolecularimaging.org) was formed about the same time with a broader focus on basic investigation at the cellular and molecular level and recently conducted its first International Scientific Symposium in Boston. The purview of both groups reaches all relevant imaging modalities and features efforts to develop and deploy novel targeted contrast agents.

Government agencies have begun to offer a variety of funding opportunities. For example, the initiation of the BECON by NIH was followed by a Congressional mandate to establish a new National Institute for Biomedical Imaging and Bioengineering devoted to development of novel technologies including molecular imaging and therapeutics, without the traditional restrictions of hypothesis-driven research. The National Cancer Institute has taken a leadership position in molecular imaging with the advent of training and research programs such as the Centers for Molecular Imaging, R&D contracts for cancer imaging agents through the unconventional innovations programs (UIP's), and preclinical drug development programs (DCIDE) for novel molecular imaging agents. The National Nanotechnology Initiative (see M.C. Roco statement at http://www.nano.gov/roco_vision.html) intersects most federal agencies in its effort to organize the new "small scale" thinking and includes the National Science Foundation, National Institutes of Health, Department of Energy, Department of Defense, Department of Agriculture, Central Intelligence Agency, Nuclear Regulatory Commission, National Aeronautics and Space Administration, National Institute of Standards and Technology, Environmental Protection Agency, and Food and Drug Administration, among others.

In Europe, multinational initiatives at the European Union (EU) level have emerged, although country-level support has existed longer and is increasing. The Sixth Framework Programme for research (2002–2006) was adopted by the EU's Council of Ministers on 3rd June 2002, and will explicitly cover clinical research. EU funds will be used to assess new therapies or validate molecular targets for cancer diagnosis and treatment. A total $2 billion over 3 years is devoted to research initiatives including "life sciences, genomics, and biotechnology for health" and "nanotechnologies and nanosciences." Large multi-component partnerships are featured including corporations, academia, and health care industries, leading to centers of excellence.

Manufacturers of imaging equipment are cognizant of the promise of the solution of the human genome to expand the targets for imaging and drug development and delivery. Corporate research programs in molecular imaging have been initiated by major manufacturers such as General Electric, Philips Medical Systems, and Siemens, among others. In some cases these programs have been developed in concert with programs in molecular diagnostics (e.g., gene chips), or in partnership with smaller biotech startups or academic laboratories that are active in the field. Traditional contrast agent developers also have begun internal efforts to produce diagnostic agents for molecular imaging, and are concentrating in particular on nuclear/PET imaging of cancer. Many pharmaceutical industry research groups already have installed impressive imaging facilities comprising all clinically applicable modalities to assist with selection of candidate agents and early

drug development. The need for new biomarkers of disease and surrogate endpoints for drug trials is driving these efforts.

THE FUTURE

Nanoscale science will play a fundamental role in imaging, biosensors, biomarkers, self-assembling tissue implants, and drug delivery over the next decade. Ironically, as devices and agents become smaller, they will require bigger and more multidisciplinary teams to realize the anticipated revolution. In contrast to the time-honored models of academic collaboration among highly focused laboratories, nanoscience efforts will require that investigators learn each other's languages and form partnerships that integrate individual intellectual components into a cohesive team approach. The complexity of the new nanotechnologies and the scope of their clinical and commercial applications require direct and immediate access to diverse "in house" expertise, which could dramatically impact the traditional academic paradigm for doing science.

The United States National Nanotechnology Initiative (see M.C. Roco statement at http://www.nano.gov/roco_vision.html) recognizes that "the relative arrangement of the elementary blocks of matter into their assemblies leads to new properties and functions even for the same chemical composition." Funding in excess of $700 million is scheduled for FY 2003 for Federal investment in nanoscale science, engineering, and technology. The chemistry, engineering, physics, and biology all will require new syllogistic reasoning to deal with interactions at this scale. It is clear that much of the basic science and its methods will be fresh, and that the practical translation of these efforts will prove more challenging than is currently imaginable.

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