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Can Small Animal Imaging Accelerate Drug Development?

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Abstract Better mechanistic understanding of disease through mapping of the human and mouse genomes enables rethinking of human infirmity. In the case of cancer, for example, we may begin to associate disease states with their underlying genetic defects rather than with the organ system involved. That will enable more selective, nontoxic therapies in patients who are genetically predisposed to respond to them. Because one of the major goals of molecular imaging research is to interrogate gene expression noninvasively, it can impact greatly on that process. Most of molecular imaging research is undertaken in small animals, which provide a conduit between in vitro studies and human clinical imaging. We are fortunate to be able to manipulate small animals genetically, and to have increasingly better models of human disease. The ability to study those animals noninvasively and quantitatively with new, high-resolution imaging devices provides the most relevant milieu in which to find and examine new therapies. J. Cell. Biochem. Suppl. 39: 211–220, 2002.
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The human and mouse genomes are highly homologous [Gregory et al., 2002]. Knowledge of the complete gene sequence of another mammal will enable the construction of relevant animal models of human disease at an unprecedented rate and with high specificity. We can study those models in a variety of ways, most incorporating invasive techniques destructive to the tissue, or we can image them. With imaging, we can perform longitudinal studies on single animals without the sampling error inherent to biopsy; however, the most important attribute of imaging is the provision of structural and functional information under physiologic conditions, mimicking the situation observed in the clinic. Molecular imaging can hasten drug development at the target identification and validation stages, in the synthesis and optimization of drug candidates, and in pre-phase I to phase II clinical trials, i.e., at almost any point in the process. It provides the link between in vitro studies and those performed in vivo, in humans. Many fine reviews of the use of human imaging in drug development exist [Gibson et al., 1993; Rubin and Fischman, 1997; Fowler et al., 1999; Hietala, 1999; Vaalburg et al., 1999; Paans and Vaalburg, 2000; Aboagye et al., 2001; Brady et al., 2001; Gupta et al., 2002], and an entire issue of *The Journal of Clinical Pharmacology* has recently been dedicated to that topic [Eckelman et al., 2001], so this review will focus on the use of small animal imaging for that purpose. Hume and Myers [2002] have recently reviewed small animal imaging with positron emission tomography (PET) in drug development.

WHY SMALL ANIMAL IMAGING?

Small animal imaging includes mainly rodents, but also involves birds, snakes, and other animals with a head or body diameter < 5 cm. Because they are accessible, easy to maintain, have a short reproductive cycle and, more recently, are easily genetically manipulated, the mouse remains the premier animal model for biomedical research. In the 40 years since the discovery of the nude mouse, researchers have learned not only how to grow tumor xenografts orthotopically, but also to produce animals that develop tumors de novo or even

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fluoresce, at will. Approximately 25 million mice are used in biomedical research annually, representing over 90% of all mammalian studies [Chatziioannou, 2002]. Over 3,000 knockout strains are currently available and among knockouts, conditional knockouts, transgenic, and mutant mice, there is essentially no limit to the diversity of strains able to be produced to study gene function. Perhaps, the most obvious reason to image those mice is to learn about the effects genetic manipulation has on each strain, particularly since unexpected phenotypes arise. Imaging with X-ray computed tomography (CT), magnetic resonance (MR), ultrasound (US), optical and radionuclide techniques can provide a nearly complete phenotype, structurally, functionally, and longitudinally, of a genetically altered animal. Other reasons to image (rather than dissect) mice are to decrease the number of animals needed per study, with concurrent decrease of statistical variance, since each animal may serve as its own control; to uncover biochemical pathways, for example, signal transduction cascades and protein–protein interactions, as they occur in vivo, in mammals; and to develop new diagnostic and therapeutic agents.

Small animal imaging began as merely another technique to answer questions posed in academic laboratories, but is rapidly becoming an industry. Several commercially available, miniaturized imaging systems for each modality and in-house molecular imaging capabilities are available at several pharmaceutical companies and equipment manufacturers worldwide. Dedicated scanners are needed because the size of the regions to be imaged in small animals can be smaller than the resolution of clinical scanners, particularly for radionuclide applications; high-resolution imaging systems can be built with sensitivities on a par with clinical instruments; and, dedicated animal devices do not detract from clinical throughput, allowing continuous laboratory investigation to proceed. Although, such dedicated systems tend to be housed in radiology departments, where adequate shielding from the high magnetic fields employed and proximity to a cyclotron are available, increasing use of commercially available ^{18}F-fluorodeoxyglucose (FDG) as a tracer, table-top optical imaging systems, and portable ultrasound machines encourage the use of these imaging systems as standard laboratory equipment in any department concerned with molecular imaging research.

SMALL ANIMAL IMAGING IN DRUG DEVELOPMENT

The most important step in the arduous process of drug development is target identification (Fig. 1). Proteins mediate function and therefore are the primary targets in drug development. As we have entered the post-genomic era, the identification of therapeutic targets is no longer a bottleneck in the drug development process. Because of alternative splicing and post-translational modification of the 30,000–40,000 genes identified in the human genome, the number of potential targets is in the hundreds of thousands, up from the mere hundreds of potential targets under study before the sequence became available. Those potential targets must be prioritized so that the 10,000 or so that are clinically viable can be prioritized further and validated.

Target validation occurs through a number of proteomics methods including two-dimensional gel separation of protein mixtures followed by mass spectrometry (2D-MS), transcriptional profiling of mRNAs, and functional screening [Kreider, 2001]. Biochemical screens for small molecule binding and enzymatic assays can be performed and have been automated on microarrays [MacBeath and Schreiber, 2000]. Antisense approaches can also be applied, where the gene that encodes the protein of interest can be shut off when the complementary oligonucleotide binds to its DNA sequence. Cellular function can then be assessed in the absence of function of that protein. An extension of that process to the in vivo case involves the use of knockout mice, where homologous recombination enables deletion of the gene that produces the protein of interest and the effects of the absence of function can then be determined in an animal model. Although surprising phenotypes occasionally arise, calling into question the validity of such models, their use remains a powerful, new method to assess protein function and is gaining increasing use not only in drug but also in radiopharmaceutical development [Pomper et al., 2000]. Screening for the function of intracellular proteins employs protein–protein interaction mapping, a new technique that enables a better understanding of cellular events including signal transduction and drug

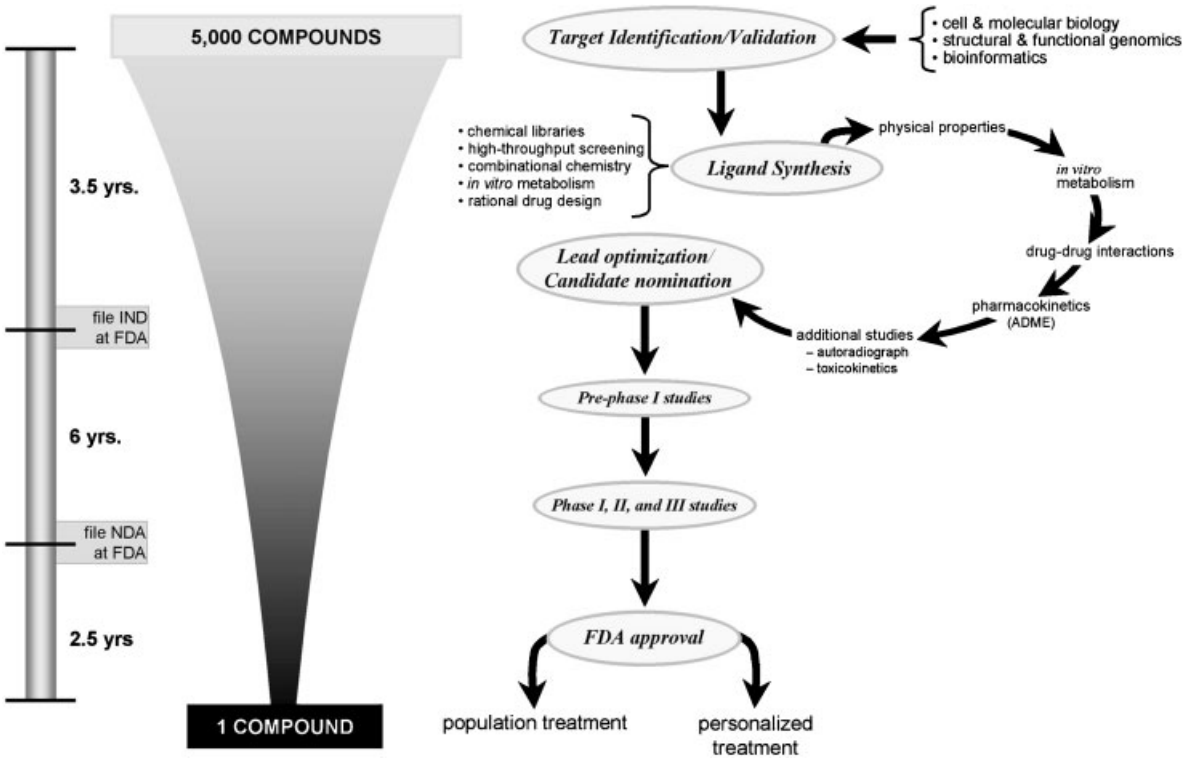

Fig. 1. Drug development.

susceptibility [Kreider, 2001]. The mapping of protein–protein interactions is also a current goal in molecular imaging research [Contag and Ross, 2002; Luker et al., 2002].

The target identification/validation stage is commensurate to the elucidation of pathophysiology of the underlying mechanism under study, for example, apoptosis during development or cancer therapy, HIF1α expression during hypoxia, activation of the NFAT pathway during immunological challenge, or overexpression of the prostate-specific membrane antigen in prostate cancer. The vast number of new targets enables the development of therapeutic agents of unique specificity, such as imatinib mesylate (Gleevec), which takes advantage of the Bcr-Abl pathway in the treatment of acute leukemia [Druker et al., 1996]. For validation, those specific targets require specific functional assays, the most physiologically relevant of which are available through molecular imaging.

The screening of protein function can be avoided if the structure is known. Structure can be determined by nuclear magnetic resonance (NMR) spectroscopy or, more recently, through high-throughput X-ray crystallography, i.e., methods of structural genomics [Montelione, 2001]. Homology modeling and computational docking methods also enable a guess at protein structure that, once known, permits the rational synthesis of suitable ligands. Dynamic combinatorial chemistry and computer-assisted drug design are finding increasing use as well. Once compounds of acceptable affinity and selectivity are identified, their physical properties, i.e., solubility, pK_a and logP, are determined. Each of those parameters is critical and can be a reason for failure of the candidate drug. Drug metabolism is then studied in human hepatocytes, if possible, or at least in hepatocytes of the species in which later toxicology studies will be performed. Drug–drug interactions are assayed and then the absorption, distribution, metabolism, and excretion (ADME–pharmacokinetics) of the candidate drug are determined in rodents and in other species. At that point, the candidate drug is ready to enter clinical trials.

Information from small animal imaging studies can be extremely helpful at the ligand synthesis/optimization stage, particularly in elucidating pharmacokinetics of the drug candidate. Pharmacokinetic imaging requires

the drug candidate to be tagged in some way, usually radiolabeled, but increasingly with fluorescent labels and, for larger molecules, with magnetic labels. In almost all cases, except for radiolabeling an identical site in the drug candidate with carbon-11 for PET, one must be mindful that an analog, rather than the drug candidate, is being studied. Central nervous system (CNS) drugs can be tagged and tracked in vivo in a single animal to determine blood–brain barrier permeability. Quantitative kinetic evaluation of drug candidates can be performed with PET, allowing calculation of relevant rate constants that describe tissue extraction, receptor-specific binding, nonspecific binding, or enzyme turnover. Pharmacokinetic knowledge obtained from imaging enables continuous monitoring of the disposition of the drug candidate, not just snapshots of the plasma concentration of the unmetabolised component, which may have little relevance to the concentration of the drug candidate at the intended site of action. That is true of CNS drugs, where brain and plasma kinetics invariably diverge and for oncologic agents, where heterogenous tumor perfusion presents the lesion with an uneven or inadequate dose, not reflected in peripheral samples. Pharmacodynamic information, i.e., the effects of the drug candidate on the tissues, is also readily available through small animal imaging. Changes in blood flow critical to anti-angiogenic therapies can be assessed by PET, MR, or US. Drug–drug interactions can be studied by radiotracers designed to probe the activity of multidrug resistance (MDR) pumps under the influence of MDR modulators, for example. All of this information can be used in an iterative fashion for structural refinement of lead compounds.

Phase I clinical trials consist of determining a safe dose of the drug candidate in healthy volunteers. Before that, i.e., in pre-phase I, radionuclide imaging has been applied in dose-finding exercises, particularly in CNS applications where the receptor occupancy of drug candidates can be calculated [Grunder et al., 1997]. Because only a small amount of mass is administered with radiotracers synthesized in high specific radioactivity, no pharmacologic effect from that administration results and doses as little as 1/1,000 of the lowest initial dose in a phase I trial can be administered [Paans and Vaalburg, 2000]. In phase II, the candidate drug is administered to patient volunteers to evaluate efficacy and search for side effects. Effectiveness is further monitored in phase III, as is the presence of long-term side effects. Preclinical development takes an estimated 3.5 years while about 6 years are required for clinical trials needed to apply to the FDA for a new drug application (NDA). A further 2.5 years is required for that application to be approved. In all, about one in 5,000 drugs reaches that stage, at a cost of up to $700 million.

Imaging endpoints included in clinical trials, such as the RECIST (Response Evaluation Criteria in Solid Tumors) criteria, rely on anatomic changes to assess the efficacy of antitumor drugs, with progression defined as a 40% increase in tumor volume [Padhani and Ollivier, 2001]. But physiologic changes detected with molecular imaging techniques antedate anatomic changes and should be more sensitive in assessing efficacy. Accordingly, PET is being applied increasingly for therapeutic monitoring, particularly for cancer, where new agents may be merely cytostatic, having a minimal effect on lesion size [Hoekstra et al., 2002]. One problem in using physiologic endpoints in determining efficacy is the lack of standardization of techniques of data acquisition and analysis, which, in the case of clinical PET, extend from measuring the standardized uptake value corrected for body surface area (SUV_{BSA}) to full kinetic modeling. Agents other than FDG, such as 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT) [Dittmann et al., 2002] and MS-325 (AngioMARK) [Bluemke et al., 2001], a blood pool MR contrast agent, are beginning to see clinical use, the latter in phase III trials. Although, those agents are able to be tested in humans directly, input from small animal imaging during early clinical trials can refine the imaging protocols, and answer unforeseen questions related to pharmacokinetics, for example, because performing animal studies is much more efficient.

SMALL ANIMAL IMAGING IN DRUG DEVELOPMENT: EXAMPLES

The standard, clinical modalities of CT, MR, US, and radionuclide imaging are available to small animals. Optical imaging is available clinically to a limited extent, but is emerging as a key modality for imaging rodents because like US, it provides an essentially instantaneous readout on portable, inexpensive

equipment. Scaling down of each modality to serve small animals is an important issue, because careful knowledge of the scaling factors involved, which are not necessarily linear, is necessary to assure an appropriate experimental design. The modalities are complementary in information, vary in anatomic and temporal resolution, sensitivity, cost, and other factors [Weissleder, 2002]. Table I synthesizes those factors as they apply to drug development. Several generalizations are possible: contrast agents enhance the utility of all modalities; CT and MR are used primarily for anatomy and pharmacodynamics; US is used for pharmacodynamics of vascular agents; and optical and radionuclide agents image gene expression, enzymes, receptors, and transporters. The sensitivities of the systems for detecting molecular events, of paramount importance in drug development, can roughly be described as: optical > radionuclide $\gg$ MR > US $\geq$ CT.

MR tissue characterization relies on the detection of water protons through the application of radiofrequency pulses to the animal in a magnetic field. Differences in the microenvironment of those protons between tissues determine the appearance of the image. MR may be used to interrogate a variety of cellular and molecular phenomena and has been applied to drug development primarily in the context of antineoplastic agents [Aboagye et al., 2001]. Tissue pH, pO_2, and various endogenous metabolites can be determined by MR spectroscopy [Gillies et al., 2002]. Occasionally, therapeutic agents can be isotopically labeled, for example, with ^{19}F or ^{13}C to be detected directly, but because MR requires concentrations of 10–100 mM for detection (compared to nano- or picomolar concentrations for PET), pharmacokinetic studies are difficult with MR, and labeled species may approach toxic concentrations. MR is particularly adept at following vascular phenomena, such as changes in blood flow that might be caused by a pharmacologic challenge.

In order to enhance the signal produced by macromolecular or cellular species to enable direct detection, several strategies have been employed, including the use of superparamagnetic particles (monocrystalline iron oxides, or MIONs) linked to the species of interest [Hogemann et al., 2000]. Because they are superparamagnetic, MIONs alter the magnetic field around them such that an obvious decrease in signal intensity is viewed on the image.

TABLE I

	Phenotyping	Pharmacodynamics	Pharmacokinetics	Gene expression	Cell tracking	Protein-protein interaction	Drug delivery	Receptor-based imaging	Cellular metabolism	Vascular Therapies	Therapeutic Monitoring	Activatable Probes for enzyme activity
X-ray computed tomography	●	●								●	●	
Magnetic resonance imaging	●	●	●	●	●		●	?	●	●	●	●
Ultrasound	●	●					●	?		●	●	
Optical				●	●	●		●			●	●
Radionuclide		●	●	●	●	●		●	●		●	

Bulte et al. [2002] used that technique to track the movement of oligodendrocyte progenitors (CG-4) in vivo over time. They used a monoclonal antibody (OX-26) to the transferrin receptor (Tfr) bound to MION that, upon binding extracellular Tfr on CG-4 cells, caused internalization of the Tfr-OX-26-MION complex enabling visualization by MR. In doing so, they were able to follow the diffusion of Tfr-OX-26-MION into the brain parenchyma of shaker rats, a model for dysmyelinating disease, after intracerebroventricular administration (Fig. 2). Therapeutics that rely on transplantation of progenitor cells, for example, to reconstitute the CNS in cases of trauma or neurodegenerative disease, will benefit greatly from MR cell tracking. Superparamagnetic particles have additional uses, including providing signal amplification for imaging gene expression [Moore et al., 2001].

A technique with excellent anatomic resolution, such as MR (50 μm for MR microscopy) can be coupled to techniques of lower resolution to study processes that generally require higher sensitivity, such as gene expression. Using an imaging reporter to visualize gene expression in vivo is an extension of using well-known histochemical reporters, such as β-galactosidase (Fig. 3). Imaging the expression of HIF-1α in cells under hypoxic stress illustrates that concept. Low oxygen tension upregulates expression of HIF-1α, which then dimerizes with HIF-1β to form a transcription factor that binds to downstream, specific hypoxia response elements (HREs) in DNA including the genes coding for the vascular endothelial growth factor (VEGF) and Glut-1. Raman et al. allowed PC-3 prostate tumors transfected with the HRE for VEGF to grow until the central core became hypoxic (Fig. 4). Using an HRE-GFP construct,

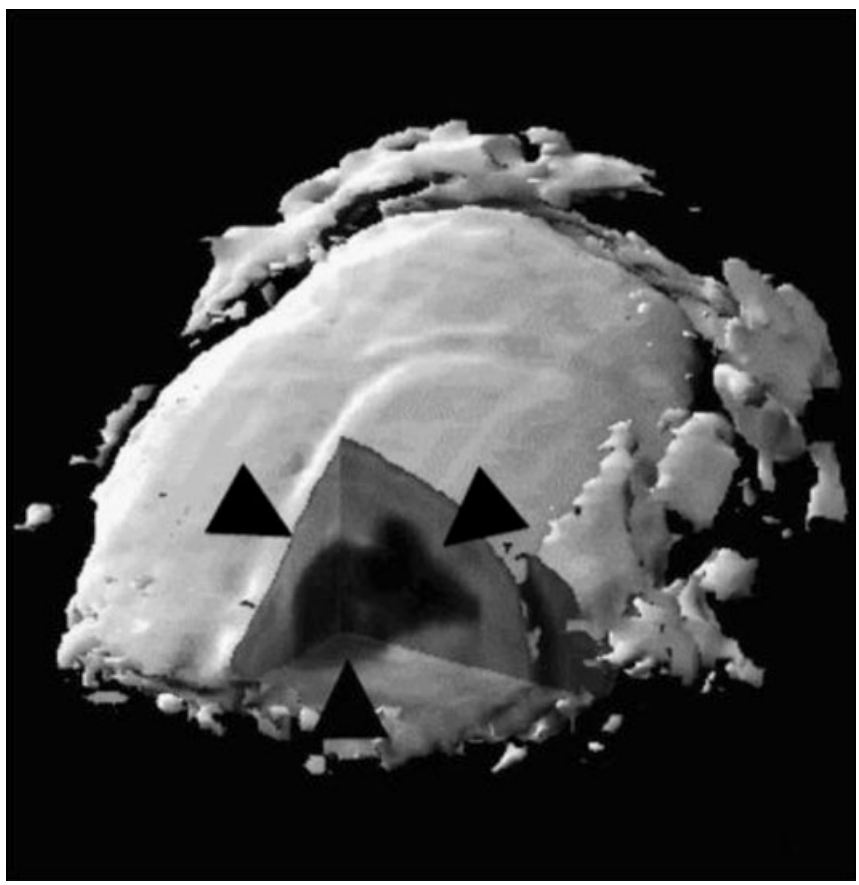

Fig. 2. Magnetic resonance (MR) tracking of CG-4 oligodendrocyte progenitors in a rat model of dysmyelinating disease (Long Evans shaker) is shown at 6 weeks after intracerebroventricular injection of magnetically-tagged cells. Note the migration of cells into the brain parenchyma (arrowheads) [Bulte et al., 2002].

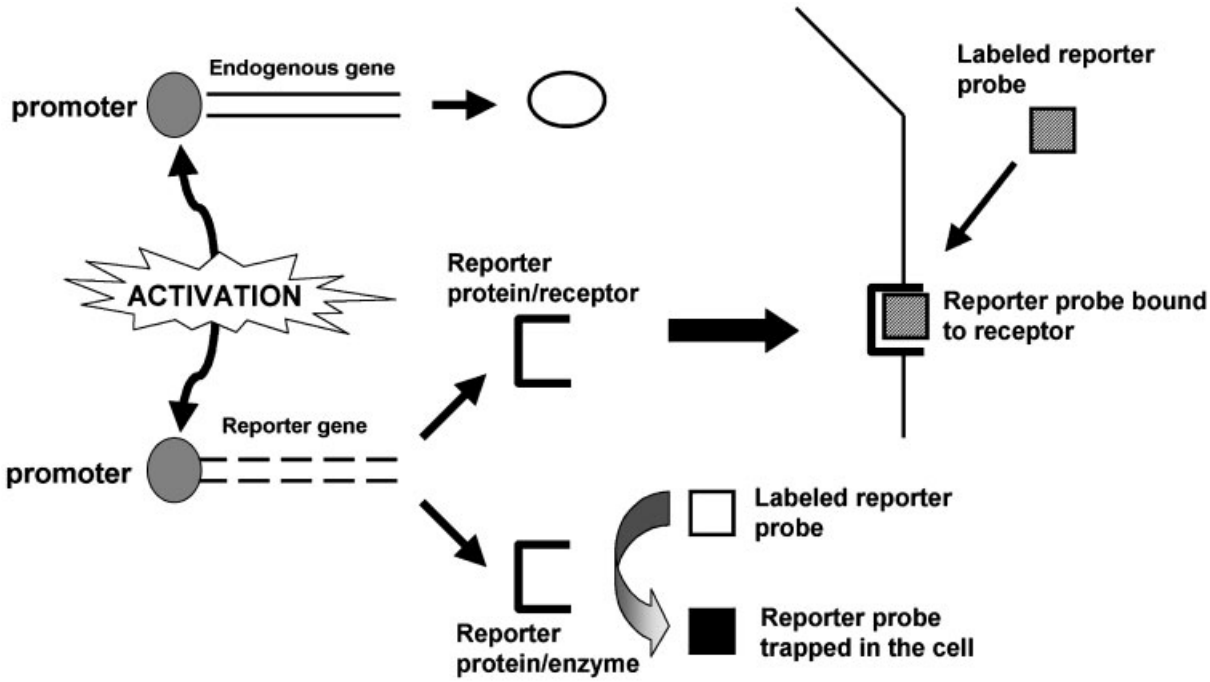

Fig. 3. Gene reporter/probe concept. Transcription of the reporter protein is linked to that of a gene of interest. The reporter may be intracellular (e.g., an enzyme such as HSV1-TK) or it may be on the cell surface (e.g., Tfr). The degree of transcription of the gene of interest (product not imageable) can be inferred because it and the gene encoding the reporter protein (product imageable) are transcribed in a 1:1 stoichiometry.

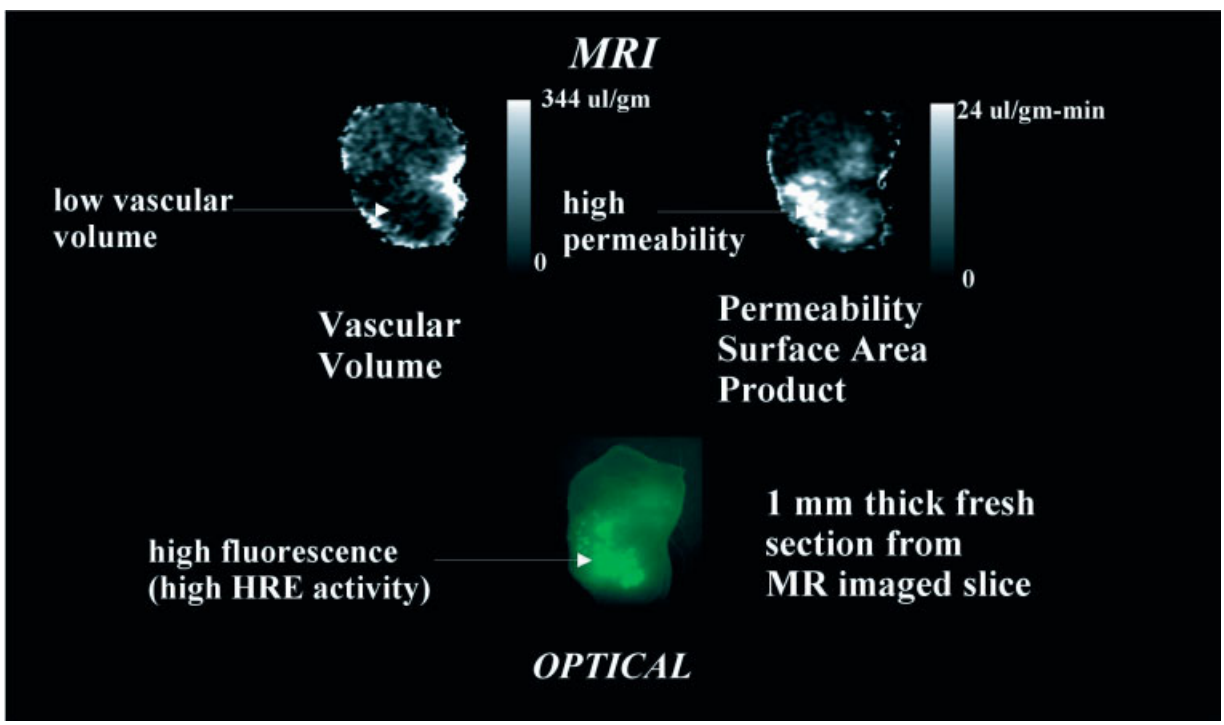

Fig. 4. Combined magnetic resonance (MR) and optical imaging. PC-3 cells stably transfected with HRE-GFP were exposed to hypoxic conditions upon tumor growth, upregulating HIF-1α, ultimately driving production of GFP.

they demonstrated activation of the HRE through optical imaging that was co-registered with vascular volume and permeability maps generated by MR. The maps showed areas of low volume and high permeability in the region of high HRE expression, as expected, since VEGF is believed to be a marker for vascular permeability [Bhujwalla et al., 2001]. Gene expression imaging to follow therapy is illustrated by a recent study by Mayer-Kuckuk et al. [2002]. They used PET with the positron-emitting HSV1-TK substrate 2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyl-5-[^{124}I]iodouracil (FIAU) to show that cells exposed to antifolates demonstrate a rapid increase in dihydrofolate reductase (DHFR) activity (Fig. 5). That is an example of drug-induced modulation of endogenous gene expression and can be used to test the effectiveness of different therapies that act through DHFR.

PERSPECTIVE

Rapid scientific advances have characterized molecular imaging and small animal imaging research over the last 5 years, but substantial challenges remain. From a technical standpoint, the development of small animal imaging devices, particularly for radionuclide applications, has not yet reached the detection limit, i.e., further improvements in sensitivity and resolution are forthcoming, although they will be incremental. Other technical challenges include: timely processing of the vast amounts of data generated by animal studies (one high-resolution mouse study can generate 75 MB of data [Weissleder, 2002]), particularly if phenotyping or other high-throughput uses of the technology are anticipated; careful validation of the imaging findings with time-tested in vitro studies, particularly for gene expression imaging; making the appropriate choices among the many available targets uncovered after sequencing the human genome; and in the synthesis of suitable probes for those targets. Synthetic chemistry capabilities remain at a premium in imaging research, an area where increased partnership with the pharmaceutical industry could help. In that regard, pharma might consider integrating functionality into their drug candidates that will enable them to provide imaging precursors as well as therapeutic agents. Availability of potential imaging agent precursors among the large databases of pharma could be made available to imaging centers, provided issues regarding intellectual

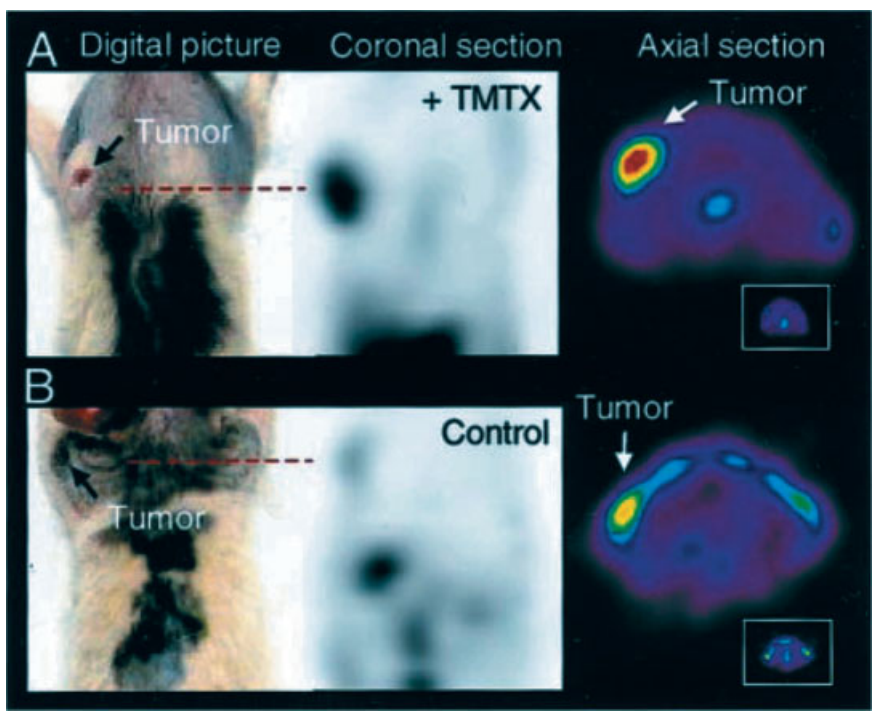

Fig. 5. Imaging endogenous dihydrofolate reductase (DHFR) induction. Antifolate treatment (TMTX) upregulates DHFR within tumor [Mayer-Kuckuk et al., 2002].

property can be adequately addressed. Many good drugs make poor imaging agents but some poor drugs, shelved by pharma, might be modified into excellent imaging agents.

Sharing intellectual property is not the only nontechnical challenge facing molecular imaging research. Gene therapy, one of the target areas of molecular imaging research, is in its infancy and has experienced several setbacks, most recently with the possibly related development of a leukemia-like illness after what appeared to be a success in treating severe combined immunodeficiency disease (SCID) [Buckley, 2002]. Also, the specific targets promised by the human genome project, each of which with the potential of an equally specific imaging agent, may not affect many people and therefore may be of limited attractiveness to pharma for therapeutic development. However, just as the Orphan Drug Act of 1983 has been implemented to address that issue, the National Cancer Institute (NCI) has implemented several initiatives to enhance the development of imaging agents, including the Development of Clinical Imaging Drugs and Enhancers (DCIDE) program.

Deeper understanding of disease processes invariably leads to more specific, less toxic therapy. Patients can now be screened genetically and segregated on that basis before beginning what could be an unnecessary regimen for someone with their genetic composition. Molecular imaging research, initially undertaken in small animals, has been aligned with important goals in therapy, particularly in cancer research, and incorporated into small animal imaging research design has been a view of translation to the clinic. Initial clinical applications of this body of work will likely involve cell tracking, using radionuclide techniques, but the long-term applications will extend to providing imaging phenotypes for patients, rendering large-scale screening processes obsolete. Drug development will benefit not only through cost savings at many pre-clinical and clinical steps, but also by improving the efficiency of research programs in pharma, which will be free to tackle more feasible and relevant targets.

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REFERENCES

Aboagye EO, Price PM, Jones T. 2001. In vivo pharmacokinetics and pharmacodynamics in drug development using positron-emission tomography. Drug Discov Today 6:293–302.

Bhujwalla ZM, Artemov D, Natarajan K, Ackerstaff E, Solaiyappan M. 2001. Vascular differences detected by MRI for metastatic versus nonmetastatic breast and prostate cancer xenografts. Neoplasia 3:143–153.

Bluemke DA, Stillman AE, Bis KG, Grist TM, Baum RA, D'Agostino R, Malden ES, Pierro JA, Yucel EK. 2001. Carotid MR angiography: Phase II study of safety and efficacy for MS-325. Radiology 219:114–122.

Brady F, Luthra SK, Brown GD, Osman S, Aboagye E, Saleem A, Price PM. 2001. Radiolabelled tracers and anticancer drugs for assessment of therapeutic efficacy using PET. Curr Pharm Des 7:1863–1892.

Buckley RH. 2002. Gene therapy for SCID—A complication after remarkable progress. Lancet 360:1185–1186.

Bulte JW, Duncan ID, Frank JA. 2002. In vivo magnetic resonance tracking of magnetically labeled cells after transplantation. J Cereb Blood Flow Metab 22:899–907.

Chatziioannou AF. 2002. Molecular imaging of small animals with dedicated PET tomographs. Eur J Nucl Med Mol Imaging 29:98–114.

Contag CH, Ross BD. 2002. It's not just about anatomy: In vivo bioluminescence imaging as an eyepiece into biology. J Magn Reson Imaging 16:378–387.

Dittmann H, Dohmen BM, Kehlbach R, Bartusek G, Pritzkow M, Sarbia M, Bares R. 2002. Early changes in [(18)F]FLT uptake after chemotherapy: An experimental study. Eur J Nucl Med Mol Imaging 29:1462–1469.

Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. 1996. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2:561–566.

Eckelman WC, Waterhouse R, Frank R. 2001. Nuclear imaging and biomarkers in drug development using approved radiopharmaceuticals. J Clin Pharmacol Suppl 41:4S–6S.

Fowler JS, Volkow ND, Wang GJ, Ding YS, Dewey SL. 1999. PET and drug research and development. J Nucl Med 40:1154–1163.

Gibson RE, Burns HD, Eckelman WC. 1993. The potential uses of radiopharmaceuticals in the pharmaceutical industry. In: Burns HD, Gibson R, Dannals R, Siegl P, editors. Nuclear imaging in drug discovery, development, and approval. Boston: Birkhauser. p 321–331.

Gillies RJ, Raghunand N, Karczmar GS, Bhujwalla ZM. 2002. MRI of the tumor microenvironment. J Magn Reson Imaging 16:430–450.

Gregory SG, Sekhon M, Schein J, Zhao S, Osoegawa K, Scott CE, Evans RS, Burridge PW, Cox TV, Fox CA, Hutton RD, Mullenger IR, Phillips KJ, Smith J, Stalker J, Threadgold GJ, Birney E, Wylie K, Chinwalla A, Wallis J, Hillier L, Carter J, Gaige T, Jaeger S, Kremitzki C, Layman D, Maas J, McGrane R, Mead K, Walker R, Jones S, Smith M, Asano J, Bosdet I, Chan S,

Chittaranjan S, Chiu R, Fjell C, Fuhrmann D, Girn N, Gray C, Guin R, Hsiao L, Krzywinski M, Kutsche R, Lee SS, Mathewson C, McLeavy C, Messervier S, Ness S, Pandoh P, Prabhu AL, Saeedi P, Smailus D, Spence L, Stott J, Taylor S, Terpstra W, Tsai M, Vardy J, Wye N, Yang G, Shatsman S, Ayodeji B, Geer K, Tsegaye G, Shvartsbeyn A, Gebregeorgis E, Krol M, Russell D, Overton L, Malek JA, Holmes M, Heaney M, Shetty J, Feldblyum T, Nierman WC, Catanese JJ, Hubbard T, Waterston RH, Rogers J, de Jong PJ, Fraser CM, Marra M, McPherson JD, Bentley DR. 2002. A physical map of the mouse genome. Nature 418:743–750.

Grunder G, Yokoi F, Offord SJ, Ravert HT, Dannals RF, Salzmann JK, Szymanski S, Wilson PD, Howard DR, Wong DF. 1997. Time course of 5-HT2A receptor occupancy in the human brain after a single oral dose of the putative antipsychotic drug MDL 100,907 measured by positron emission tomography. Neuropsychopharmacology 17:175–185.

Gupta N, Price PM, Aboagye EO. 2002. PET for in vivo pharmacokinetic and pharmacodynamic measurements. Eur J Cancer 38:2094.

Hietala J. 1999. Ligand-receptor interactions as studied by PET: Implications for drug development. Ann Med 31: 438–443.

Hoekstra CJ, Hoekstra OS, Stroobants SG, Vansteenkiste J, Nuyts J, Smit EF, Boers M, Twisk JW, Lammertsma AA. 2002. Methods to monitor response to chemotherapy in non-small cell lung cancer with 18F-FDG PET. J Nucl Med 43:1304–1309.

Hogemann D, Josephson L, Weissleder R, Basilion JP. 2000. Improvement of MRI probes to allow efficient detection of gene expression. Bioconjug Chem 11:941–946.

Hume SP, Myers R. 2002. Dedicated small animal scanners: A new tool for drug development? Curr Pharm Des 8:1497–1511.

Kreider BL. 2001. Proteomics: Defining protein function in the post genomics era. In: Doherty AM, editor. Annual reports in medicinal chemistry. New York: Academic. p 227–235.

Luker GD, Sharma V, Pica CM, Dahlheimer JL, Li W, Ochesky J, Ryan CE, Piwnica-Worms H, Piwnica-Worms D. 2002. Noninvasive imaging of protein-protein interactions in living animals. Proc Natl Acad Sci USA 99: 6961–6966.

MacBeath G, Schreiber SL. 2000. Printing proteins as microarrays for high-throughput function determination. Science 289:1760–1763.

Mayer-Kuckuk P, Banerjee D, Malhotra S, Doubrovin M, Iwamoto M, Akhurst T, Balatoni J, Bornmann W, Finn R, Larson S, Fong Y, Gelovani-Tjuvajev J, Blasberg R, Bertino JR. 2002. Cells exposed to antifolates show increased cellular levels of proteins fused to dihydrofolate reductase: A method to modulate gene expression. Proc Natl Acad Sci USA 99:3400–3405.

Montelione GT. 2001. Structural genomics: An approach to the protein folding problem. Proc Natl Acad Sci USA 98:13488–13489.

Moore A, Josephson L, Bhorade RM, Basilion JP, Weissleder R. 2001. Human transferrin receptor gene as a marker gene for MR imaging. Radiology 221:244–250.

Paans AM, Vaalburg W. 2000. Positron emission tomography in drug development and drug evaluation. Curr Pharm Des 6:1583–1591.

Padhani AR, Ollivier L. 2001. The RECIST (Response Evaluation Criteria in Solid Tumors) criteria: Implications for diagnostic radiologists. Br J Radiol 74:983–986.

Pomper MG, Musachio JL, Scheffel U, Macdonald JE, McCarthy DJ, Reif DW, Villemagne VL, Yokoi F, Dannals RF, Wong DF. 2000. Radiolabeled neuronal nitric oxide synthase inhibitors: Synthesis, in vivo evaluation, and primate PET studies. J Nucl Med 41:1417–1425.

Rubin RH, Fischman AJ. 1997. Positron emission tomography in drug development. Q J Nucl Med 41:171–175.

Vaalburg W, Hendrikse NH, de Vries EF. 1999. Drug development, radiolabelled drugs and PET. Ann Med 31: 432–437.

Weissleder R. 2002. Scaling down imaging: Molecular mapping of cancer in mice. Nat Rev Cancer 2:11–18.