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Animal models for imaging

Barbara Y. Croft*
Biomedical Imaging Program, National Cancer Institute, National Institutes of Health, Rockville, MD 20852, USA

Abstract. Animal models can be used in the study of disease. This chapter discusses imaging animal models to elucidate the process of human disease. The mouse is used as the primary model. Though this choice simplifies many research choices, it necessitates compromises for *in vivo* imaging. In the future, we can expect improvements in both animal models and imaging techniques.

1. Introduction

Animal models can be used in many ways in the study of disease. The underlying idea of this chapter is that animals used as models of human disease allow us to image that disease process. Further, the animals can be adapted especially for imaging, or the experiment can benefit especially from the use of imaging during the course of the protocol. This chapter explores the use of animal models for imaging. The concentration is on whole, live animals, not *in vitro* or post-mortem experiments. Cancer is the disease focus, but the methods generally are applicable to other diseases and other types of research, such as those focused on the heart or brain.

Using animal models in the study of disease allows the use of techniques and simulations that are not feasible in human beings. Animal models permit the initiation of the disease process and allow the investigator to follow the process to its natural conclusion, with frequent sampling through the course, going far beyond what is possible with cell cultures. Animal models allow the application of drugs for treatment and the titration of drug doses over time, along with the study of side effects of the drug, and permit better estimation of side effects in humans and of the amount of drug that is needed to produce a therapeutic effect on the disease process. The aim is to create a model that mimics the human course as closely as possible, except for the truncation of time allowed by the shorter life span and higher metabolism of the animals. The standard methods used to follow the course of human disease – such as sampling body fluids, histopathology, and necropsy – are employed, along with others – such as chronic instrumentation – that cannot be used in humans. Naturally, the small size of some animal models may make the application of some techniques more difficult, but researchers have been amazingly creative in the methods used to overcome the problems of small size and truncated life span.

Animal models also permit manipulation of genes to create animals with particular susceptibilities. Natural mutations may be observed and utilized for research purposes. Genetically different animals may better simulate a particular disease process and also allow the researcher to follow the implications of a genetic modification to its final effects, which may provide insight into human examples of similar mutations.

There is much discussion about the choice of the animal model, and the range of possibilities is wide. Flies are a favorite of geneticists; they have been used for brain metabolism and function studies employing imaging as well [1,2]. Surgeons prefer dogs because they are resilient mammals with anatomy similar to humans and because they are large enough for techniques used on humans. Going beyond dogs to larger farm animals is out of the ordinary but is practiced for special reasons. Sheep are a good model for the placenta, for example, and cats often are used in neurological research. For yet other research, wild animals and am-

*Corresponding author: Biomedical Imaging Program, National Cancer Institute, National Institutes of Health, 6130 Executive Boulevard, EPN 6064, Bethesda, MD 20892-7412, USA. (FedEx: Rockville, MD 20852-4910). Tel.: +1 301 496 9531; Fax: +1 301 480 3507; E-mail: croftb@mail.nih.gov.

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phibians, including woodchucks, frogs, toads, and fish, are used [3]. Non-human primates are used when lower animals fail the test of similarity to the human system, but larger primates are difficult to raise and keep and therefore are expensive.

Over time, criteria for the choice of animal models have evolved based on the question at hand. The better models are developed for the use of many people in an evolving science, with improvements in the model coming before and following advances in scientific knowledge. The criteria that guide investigators in the choice of model include: the human disease under consideration; the similarity of the organ or system of interest to the human counterpart; the similarity of the whole disease process, from genetic changes through disease expression and progression to the human counterpart; timing of the model's life stages; and the economics of the situation, including the difficulties of keeping the animal, regulations, and costs of care and feeding. As we proceed through the discussions of animal models in this and other chapters, readers may question how well the listed criteria are complied with.

The above-listed criteria imply a characterization process for a model that will allow users to make a decision about the soundness of the application of the model in the intended circumstances. In some circumstances, for example, genetic similarity of the disease process may be more desirable than the similarity of expression of the disease process. This might be the case when a therapy targeted to a particular pathway is being tested; the pathway of interest must be expressed or over-expressed in the disease process and, preferably, not expressed in normal cells.

Because the preparation and characterization of a good model requires time and energy, consideration must be given to the properties desired or the effort will be misspent. There are blind alleys in science, but careful consideration beforehand may prevent the expenditure of great effort in vain. The choice and development of a model by an entire research community involves even greater time and effort but is rewarded by the basic work being available to the entire community. In addition, the greater the use of the model, the greater the gain for everyone. Characterization of the models and publication of the results benefits the entire community and produces a synergy for the science.

Rodents as a group have been favorites of the research community because they are mammals, do not have a large advocacy group (because although they may be kept as house pets, they are very common and often are seen as a nuisance), have shorter life cycles, and are not too fierce. The commonly used rodents are mice, rats, rabbits, and guinea pigs. Rodents are used widely in drug testing, although the US Food and Drug Administration often requires that testing be performed in two species – one not a rodent – because of the similarity of behavior among rodent species in test circumstances.

Mice have been chosen by cancer researchers and many others as the preferred model. Mice typify similarity to humans, are fairly docile and easy and inexpensive to raise and keep, have short gestation times and life cycles, and are amenable to genetic manipulation. Mice were one of the animals chosen for gene mapping because they were in such wide use. The National Cancer Institute (NCI) chose to highlight mouse modeling of human cancer by establishing a consortium of 21 members, the Mouse Models of Human Cancer Consortium (MMHCC), to develop mouse models for a number of human cancers. The current status of the consortium's efforts can be viewed at http://emice.nci.nih.gov/. The contents of the Web site include a series of review articles on the state of the science in the cancers under consideration, including the results of a series of workshops held by histopathologic experts in each tumor area that compared mouse and human histopathology. The histopathology effort is described by Galvez et al. [4]. A repository of the mouse models supported by the consortium members has been established at the NCI Frederick Laboratory; at this writing there are 50 strains, with breeding pairs available to investigators upon the signing of a Material Transfer Agreement. The strains' descriptions are available, along with a citation for the scientific paper that describes the strain. Also included are a series of "tools", including microarray data, cancer models, reference images, a histology database, and mouse model publications. Other National Institutes of Health (NIH) Web sites provide additional information about mouse genetics, such as that of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the Cancer Genome Anatomy Project (http://cgap.nci.nih.gov/), which contains human and mouse genomic data, including expressed sequence tags (ESTs), gene expression patterns, single nuclear polymorphisms (SNPs), cluster assemblies, and cytogenetic information; informatics tools to query and analyze the data; and information on methods and resources for reagents developed by the project. An article for laymen on the use of mice in cancer research is available at http://www.vetmed.ucdavis.edu/Animal_Alternatives/cancer.htm. Work continues on the laboratory mouse

and rat. As the MMHCC and other mouse modelers continue their work, the amount of information is expected to increase. The Trans-NIH Mouse Initiative (http://www.nih.gov/science/models/mouse/) is an attempt to bring together information about all NIH mouse modeling initiatives.

The National Human Genome Research Institute (http://www.genome.gov/) has finished the sequencing of the genomes of the bacterium Escherichia coli, Saccharomyces cerevisiae (commonly known as baker's yeast), Drosophila melanogaster (the fruitfly), and Caenorhabditis elegans (the multi-cellular roundworm).

There are a number of ways in which mice are not the ideal model animal. Mice are very similar genetically to humans, but their genes are arranged differently on their chromosomes. As a result, mutations that depend on nearness of position on a chromosome are not the same in the two species. Anatomically, mice are not little humans, either. For example, the mouse prostate is quite different anatomically from the human prostate, which means that experiments depending on their similarities must be planned carefully. The differences also have caused a mental problem for investigators: structures in mice and humans have different names; we even name front and back and head and tail differently in animals that stand upright than for those that run on four legs. Thus, any comparative scheme must account for these differences.

The size of mice poses a problem for imaging and surgery. Many of the imaging techniques were developed with adult human size in mind, so there are problems for pediatric imaging. For a number of imaging modalities, miniaturization of the equipment is possible and results in images of superior resolution. For others, the transfer has not, as yet, been made so successfully. Mouse fur poses problems for optical imaging techniques, since most experimental mice are not nude. In addition, the techniques that accompany surgery and may be required for imaging and post-surgical care – such as administering anesthesia; and monitoring oxygen saturation, body temperature, heart rate, and blood pressure – must be miniaturized for use with small animals. Mice must be restrained for imaging, which usually has required anesthesia during imaging. Mice, especially mice weakened by genetic manipulation or disease, may die from anesthesia.

2. Use of animal models in the study of cancer

Animal models are used in the study of cancer and other diseases. Since there is so much concentration on the mouse, the mouse is the animal of choice for the development of small animal imaging equipment.

The simplest model of human cancer that has been developed in the mouse – and the de facto standard today – is the subcutaneous flank model, in which tumor cells are injected under the skin of the mouse flank and allowed to grow. At times, both flanks, and perhaps both shoulders, are used so that the animal can be its own control. Versions of the tumor cells can then be used in the same overall environment. There are issues with this model, however, in spite of its acceptance. Injection of human tumor cells requires the use of an immune-compromised mouse, such as the nude or SCID mouse. The subcutaneous location is easy to observe, palpate and measure, but it is not typical of the environment in which those tumor cells originate and grow. The vasculature and extracellular fluids differ, so delivery of chemotherapeutic agents may be very different from that of the native tumor site. Drug testing in the flank model often has yielded promising results that were not borne out in subsequent human testing; this may be due to the tumor environment in the flank model. Also, subcutaneous model tumors rarely metastasize, so they are not good models of disease progression in human cancer. If mouse tumor cells of a similar type to the human tumor are used in the flank model in an immune-competent mouse, the tumor itself may not exemplify the human tumor.

Imaging of animal tumors may be accomplished by all of the ordinary techniques for imaging human tumors, as described in the chapters of this volume. Of particular utility are magnetic resonance imaging (MRI) and positron emission tomography (PET) imaging for following tumor development and progress of therapy. Another technique is to label the tumor cells for optical imaging with a fluorescent protein so that the tumor is visible under special lighting. Optical techniques have become popular because they require less expensive equipment and less animal preparation than do the more instrument-intensive MRI and PET.

The standard methods for using x-ray, MRI, and PET in humans involve imaging them as they are or adding a contrast agent, be it iodinated x-ray contrast agent, a gadolinium-ion-based MRI contrast agent, or a radioactive material for PET. In many experiments, the animals are imaged a number of times over a period of weeks to ascertain the stage of the disease and possibly the success of therapies. X-ray CT is used to discover mutations in mouse populations [5], for diagnosis, and to follow therapy [6].

In an imaginative experiment, the group at Sherbrooke looked at the near-term progress of therapy in

an animal model with an optical method [7]. Their experiment mirrors techniques by which many cancer researchers think it may be possible to discover whether a given therapy will be effective in a given patient. Implanted mammary tumors in mice were treated with a photosensitizer and then exposed to light. F-18 fluorodeoxyglucose was injected to study the metabolism of the tumors after therapy. The results showed that the tumor uptake of the radioactive tracer after 15 minutes reflected the tumor metabolism and could demonstrate the relative effectiveness of the photosensitizer. In another experiment, Weissleder's group showed that matrix metalloproteinase (MMP) inhibition can be imaged directly within hours of therapy initiation using near-infrared probes [8,9]. The University of Michigan group used diffusion MRI to view changes in the apparent mobility of water in a tumor after therapy to permit the prediction of therapeutic outcome [10]. In contrast, cancer patients undergoing therapy usually are assessed weeks after treatment using anatomic information, rather than immediately after therapy using functional information. A rat model also was used in the study of microvessel density by MRI using a new blood-pool contrast agent [11].

3. Creation of animal models specifically for imaging purposes

As the mouse modelers create mice with specific cancers as models of human cancer, imaging researchers have sensed that it may be possible to create a model animal with specific characteristics for imaging. The genetic changes required for imaging then may be combined with those required to create the cancer model so that an aspect of the cancer could be imaged as part of an experiment with the mice. Research reported in the literature combines the development of techniques necessary for transfecting cells and creating mouse models with the development of imaging techniques. Because this review concentrates on imaging, the segment of the literature consulted for this review is the imaging segment; not the genetic manipulation segment. Readers should look elsewhere for the fine points of mouse genetic manipulation.

The threads of this discussion of the use of genetic manipulation to add imaging to an experiment are several. One thread starts with the genetic manipulation of cultured cells to fit them for imaging, while another thread starts with manipulation of the animal to facilitate imaging. Both threads have their roots in experiments with cultured cells to prove the steps in the hypothesis. Of course, imaging usually is not an end in itself, so the uses to which imaging is put reflect, first and foremost, the research interest of the investigator and, secondarily, the possibilities of the technique.

A widely used method involves the use of green fluorescent protein (GFP). The history of the use of fluorescent proteins goes back to observations of fluorescence in marine creatures. Harnessing this emission for purposes other than the measurement of calcium ion changes has been relatively recent. Cell cultures and animals can be grown with GFP in selected cells. Other native and altered fluorescent proteins have been used and allow the possibility of experiments with several effects at once using different colors [12–14]. Apparently, the addition of GFP to the genetic material in a cell does not affect the hardiness of the cell and the intent of the experiment. Of course, the preparation must be tested to be sure the fluorescent protein and its activation and measurement will not affect the system under consideration [15–18].

Hoffman and AntiCancer, Inc., have developed a number of fluorescently labeled tumor cell lines [19, 20] for use in experiments in which tumor tissue is transplanted in very exacting surgery into close proximity to the native location of a particular tumor variety in mice. These orthotopic model mice can be used in experiments for testing therapeutic drugs. As the tumor grows and perhaps metastasizes, progress can be recorded with images; the mice need not be anesthetized for imaging unless the tumor tissue is surgically exposed (Fig. 1). Both red and green fluorescent proteins are used. This commercial effort shows the power of the possibilities of these techniques. As the cells in the labeled cell line proliferate in the mouse, they express the fluorescence and give evidence of their presence whenever the mouse is visualized in the appropriate wavelength of light.

The Stanford group used a different idea to achieve an optical imaging end by using the firefly luminescent system, luciferin-luciferase [21,22]. The mouse tissues of interest are created with the ability to express luciferase. When the mouse is injected with luciferin, it glows in all the sites where luciferase is present. Oxygen is required but does not seem to be a limiting factor because the system has been used in relatively hypoxic situations.

The NIH group combined the luminescent and fluorescent systems in one model [18,23]. Mice were infected with a luciferase-expressing virus that infected their tumors; the particular tumor cell line used for the

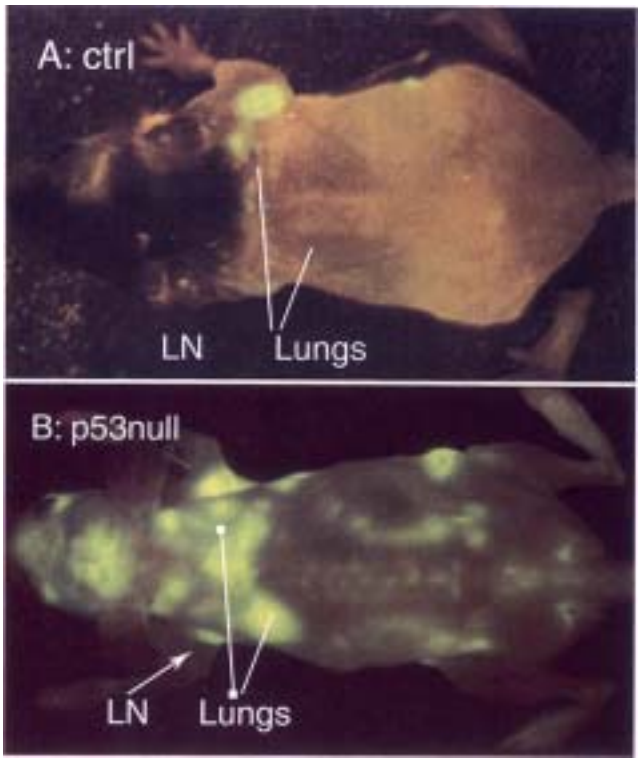

Fig. 1. Whole-body fluorescence imaging of lymphoma dissemination. Lymphomas with the indicated genotypes and transduced with a GFP-expressing retrovirus were transplanted into recipients to monitor lymphoma dissemination in a whole viable animal by GFP fluorescence. At comparable LN enlargements (e.g., axillaries LN, see arrows), the control lymphoma is restricted to the lymphoid compartment (A); while p53 null over-expressing lymphoma are much more disseminated infiltrating liver, kidneys, lungs (marked), and brain (B). (Courtesy of AntiCancer, Inc.).

demonstration expressed GFP. This group also has investigated the quantitative relationship between tumor size and GFP fluorescence (Figs 2 and 3) and used the results to quantitate treatment response.

The University of Michigan group has been developing a model system to investigate apoptosis [24].

In an example using the MRI contrast induced by iron oxide, a rat glioma cell line that over-expresses a special abundance of transferrin receptor was grown in a mouse model [25]. A conjugate of human transferrin and monocrystalline iron oxide nanoparticle (MION) was used to probe for the transferrin receptors. The experimental evidence showed that the transferrin-MION construct accumulated in the cells in large enough amounts to be imaged with MRI. The more the transferrins bound to each MION, the better the uptake and binding by cells [26].

4. Where this might lead – Diagnosis, therapy

When considering the future of molecular imaging and its applications in research and the clinic, one should consider that the gleam in someone's eye today can become the reality of tomorrow. The speed with which this occurs can be dazzling. MRI is a good example; the technique initially was developed as the nuclear magnetic resonance (NMR) of laboratory samples in the very special circumstance of spinning samples in double quartz tubes. It was applied to imaging the mouse and then the human. The changes in medical diagnosis wrought by CT and MRI have revolutionized the approach to trauma and disease.

What we see in early detection right now is a combination of techniques capable of finding disease in various stages. This is true not only for cancer, but for all of the major afflictions. The public is embracing the concept of profiling oneself with a CT or MRI scan at intervals; but medical researchers and insurance companies, mindful of the need for clinical trials to prove efficacy and cost benefit, have not adopted them as screening tools for the public. A number of imaging examinations, most of them yielding anatomical information, have been recommended for detection of disease before it becomes a clinical problem. Once we learn from the

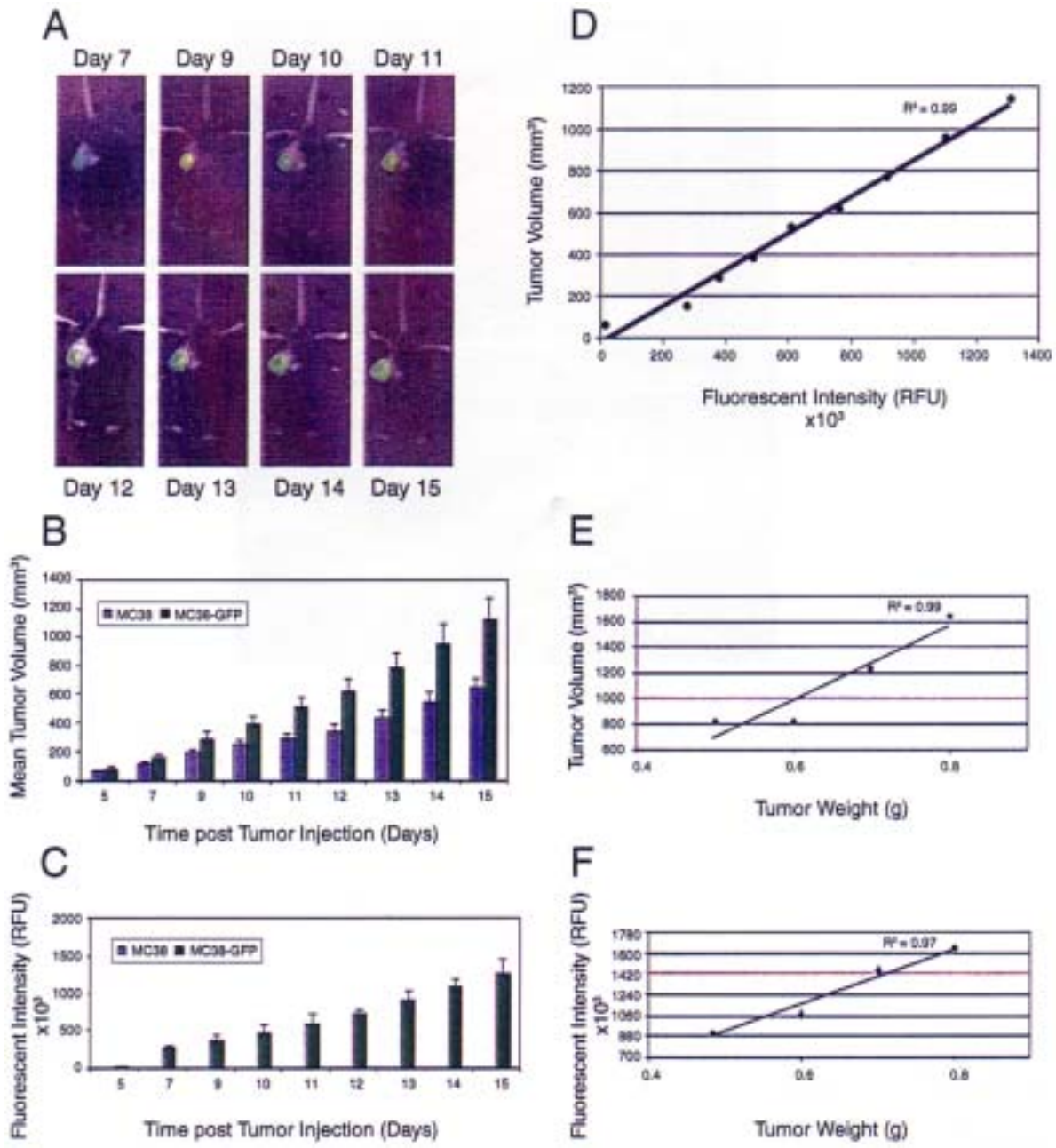

Fig. 2. Serial measurements of tumor volume and tumor fluorescence *in vivo*. MC38 or MC38-GFP tumor cells were injected subcutaneously into C57/BL6 mice. Non-invasive optical imaging was performed and the tumors were measured with calipers on the days indicated. A. Serial greyscale-fluorescent overlay images of a representative mouse bearing an MC38-GFP tumor. B. Serial tumor volumes as measured by calipers and calculated using the formula $V = 0.52 \times W^2 \times L$. C. Quantification of tumor-fluorescent intensity. D. The serial tumor volume and fluorescent intensities from each experimental time point were correlated. E, F. On Day 15, mice were sacrificed after measurements were obtained and the tumors were harvested and weighed. The tumor weights were correlated to tumor volume (E) and fluorescent intensity (F). $n =$ 4–5 per group. (From [18].).

animal models how to detect disease early, we will need to know more about the natural history of the disease in humans and to what extent it regresses by itself; we must avoid making the treatment for early disease a cause of other disease later.

The techniques that currently are in use will become

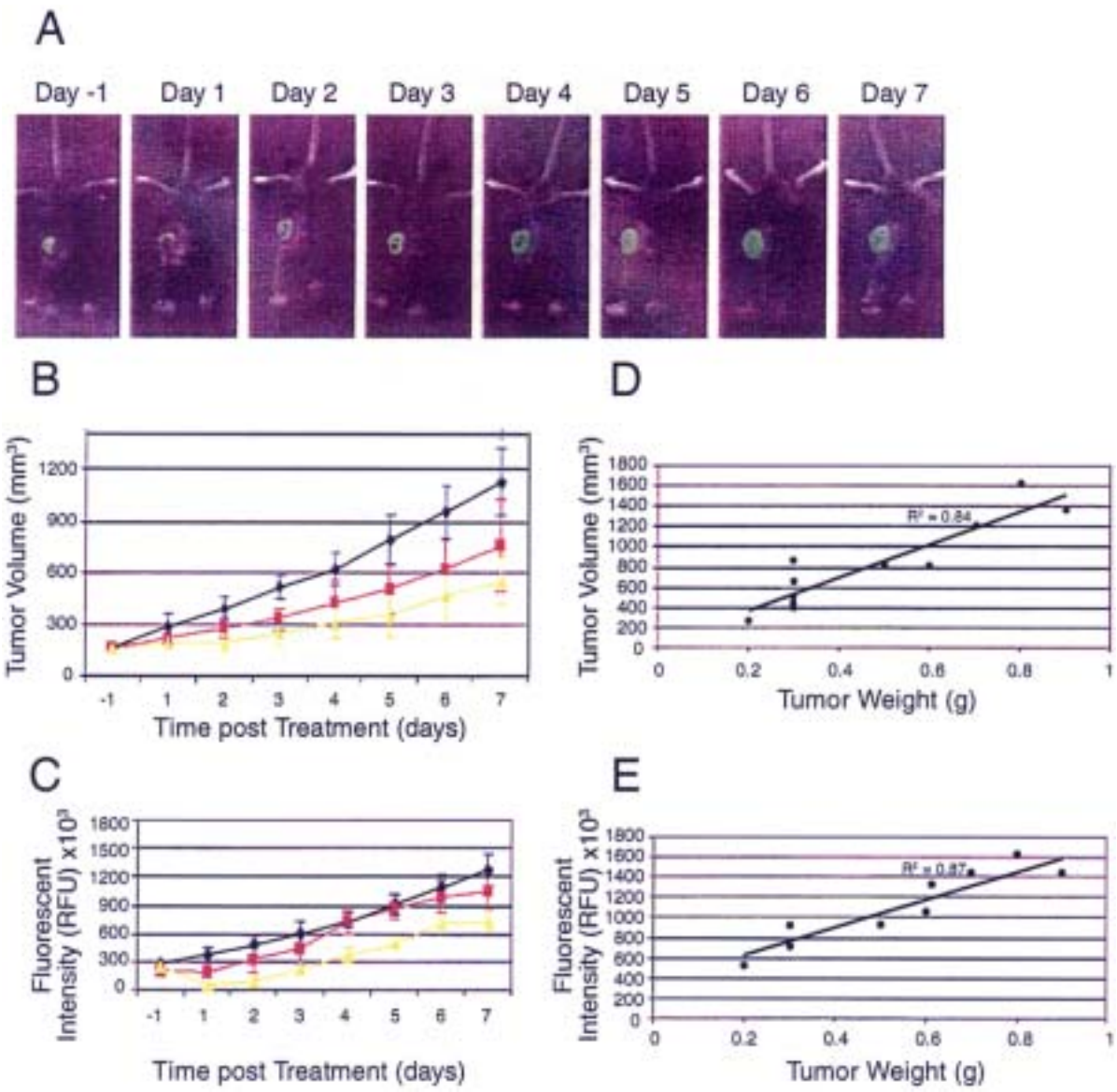

Fig. 3. Quantitative assessment of treatment responses. MC38-GFP tumor cells were injected subcutaneously into C57/BL6 mice. When tumors reached an approximate volume of 160 mm^3, mice were systemically injected on Day "0" with either PBS (control, blue lines), 2 μg TNF-a (pink lines), or 4 μg TNF-a (yellow lines). Non-invasive optical imaging was performed and the tumors were measured with calipers on the days indicated. A. Serial greyscale-fluorescent overlay images of a representative mouse treated with 4 μg TNF-a. B. Serial tumor volumes as measured by calipers and calculated using the formula $V = 0.52 \times W^2 \times L$. C. Quantification of tumor fluorescent intensity. D, E. On Day 7 after treatment, mice were sacrificed after measurements were obtained, the tumors were harvested and weighed, and the tumor weights were correlated to tumor volume (D) and fluorescent intensity (E). $n = 3$–5 per group. (From [18].).

more quantitative; the first observations always are qualitative. Evidence of quantitation comes later, after many careful experiments. Optical techniques are going through this experimental phase now. As there are many different steps and techniques, the experiments are complex. When gene transfection and expression are the foundations of a model, it must be proven stable and reliable before an entire scheme is based on it. In addition, we need to learn what the difference is between cells that show the effect and those that do not; are we seeing the heterogeneities of gene expression or some other effect?

Once the models are ready, screening and characterization of putative cancer therapeutics are the ma-

jor objectives. Characterization of the models themselves will lead to many new discoveries, which in turn will be reflected in the way we approach drug screening. There is much talk today of molecular targets and molecular pathways of disease. As we study the processes in cells, it is ever more obvious that the pathways are linked and redundant and that thinking of disease processes in terms of single targets is simplistic. The models will be very helpful in giving us a window into this multi-dimensional world.

Using x-ray imaging techniques or radioactive compounds for disease detection always poses a radiation risk. Even in animal experiments, the use of diagnostic radiation should be avoided if possible. To the extent that no substitution can be made, the radiation dose must be made as low as is reasonably achievable (ALARA). This is true of both animals and humans. Animal models have become more complicated and more expensive with regard to both money and time, so giving the animals high doses of radiation during the course of research is not acceptable. For humans, radiation never should be used idly. The whole-body CT mentioned above confers a radiation dose; if there is no concomitant benefit, then the dose not only is wasted, it is harmful.

For animals, especially small ones such as mice, there are serious issues with monitoring and maintaining their normal body physiology in the imaging situation. There also are issues of mouse colony hygiene that must be solved in imaginative ways when multiple imaging sessions are added to the protocol to which a laboratory mouse is subjected. These issues will be approached and solved, but this is not an exciting research area for many federal funders. It is hoped that it will be possible to find funding for investigators who want to find solutions to these problems.

Better and more accessible models always are needed. As each gene, pathway, and receptor is better understood, models are made in which these are expressed, not expressed, and over-expressed. This process helps us understand the setting of the disease in question. To the extent that many of our diseases may be caused by more than one gene, it will be necessary to create animal models with the same kinds of changes. It also will be necessary to test therapies in concert, not alone. We always are hoping for major synergies when combining drugs into chemotherapeutic "cocktails", but often all that is achieved is minor incremental improvement, such as a few more months of life. We must build the models to incorporate our understanding of the pathways and receptors and test the drugs we know to have therapeutic effects in these models.

The genetic changes in animal models also may cause more than one disease. Frequently, for example, substances that are over-expressed in tumor cells are the same ones that are over-expressed in cardiac disease. This also means that cooperation between disease modelers in different research areas is necessary to avoid repeating the same work. Investigators often are more aware of this (because of the necessity to seek funding wherever it may be) than are the funding agencies and disease-focused associations, which have their eyes firmly fixed on their own disease area. It would be exciting to see one of the major cancer funding agencies collaborate with a major funding agency in another disease area to sponsor a meeting or workshop on the possibilities for common approaches.

5. Applications to human cancer and limitations of animal models

In the future, expect that what is being accomplished in test tubes and Petri dishes and multiple-well plates today will be accomplished in human beings tomorrow; often using imaging methods. It is one thing to discover that a person has a particular genetic change that makes him subject to a high risk of a disease, yet another to discover by a blood or urine test that there is disease present, and still another thing to locate the site of that disease. To the extent that much disease seems not to be inherited, it may not be possible to decipher high risk in much of the population.

Optical imaging is an area that currently is being applied to array analysis and animal model imaging. In humans, the current depth of tissue from which optical signals can be obtained is too small to permit the imaging of all parts of the body. Those body parts accessible by catheter can be imaged, as can the skin and the breast. It is expected that research and more powerful computers will make imaging from greater depths possible. This will broaden the range of imaging techniques available for use in humans. Warren et al. demonstrated that detection of two-photon absorption of femtosecond-shaped pulses makes imaging from greater depths possible [27].

The reader may have been keeping score mentally of the techniques described for animal models and whether they can be applied to humans. Clearly, we are not about to evolve into a race of human beings that expresses GFP or luciferase. And, since we do not know what disease a particular individual will fall prey to, we would not know what tissues to tag. There

are, however, ways to label specific cells for follow-up. Methods that can be applied systemically clearly are preferred, but they may not permit the required specificity. One approach has been to tailor specific antibodies to the particular tissue of interest; perhaps antibodies labeled for imaging and therapy may be tailored to the tumor in an individual. This is painstaking and, thus far, has not seemed to lead to disease control, perhaps because the metastases are the result of a different cell population than is the bulk of the primary tumor. In other cases, antibodies have been made to substances that are expected to be present in the tumor type in the patient at hand; these antibodies can be used in a whole group of patients. Overall, though, growing antibodies is a hit-or-miss proposition, because we cannot control the specific point of attack of the antibody. All too often, that antigenic target also is present on other cells, so the antibodies turn out to be less specific.

In one example, a pancreatic beta-cell-specific monoclonal antibody was created that bound to and accumulated in beta cells. Pancreatic beta-cell mass is a determinant of the amount of insulin that can be secreted; in humans, beta-cell mass only can be assessed at autopsy. The monoclonal antibody was labeled with a radioactive material that permitted imaging and quantitation of the beta-cell mass in normal mice and in a mouse model of diabetes [28].

The same methods of viral delivery by vaccinia or by a non-toxic herpes simplex virus (HSV) – either directly to the tumor bed or systemically, depending on the specificity of the virus – are possible for delivering substances that will permit later imaging, just as they can deliver therapies or substances that later will permit therapies. HSV-thymidine kinase (TK) often is used for such purposes, targeting TK receptors, which makes possible the use of anti-HSV drugs as imaging and targeting agents [29,30].

"Smart" probes are another way to utilize the living system to create a beacon to advertise the presence of a substance. Investigators have used enzymes that are over-expressed to cleave or otherwise chemically change reporter substances; the original substance has no signal, while the chemically activated one announces the presence of the enzyme [31]. This idea also can be used to probe for calcium [32] or pH differences [33].

Delivery of drugs and other therapies to their targets always is an issue. Therapeutic drugs are tested in animal models to find the area under the time curve for the drug, so that the dose for therapeutic efficacy can be sustained. More careful study will yield information about the area under the time curve in the tumor; often the doses are higher and more sustained in other organs, leading to dose-limiting side effects. Gene therapy especially must be delivered to its target, since the effect of the treatment depends on the location of a transfected gene in the cells of interest. The imaging methods described in this volume particularly are suited to exploring such localization.

The notion that a period of weeks and perhaps even several courses of treatment must elapse before a patient can be assessed for treatment efficacy and that anatomic methods – such as x-ray CT, are best for this assessment – can be banished in favor of immediate assessment with the first and perhaps successive treatments [7,8]. However, it seems likely that, if an imaging study can give evidence of treatment effectiveness within hours after treatment, a blood or urine test could give the same evidence. What the imaging study can do in addition is to show that parts of the tumor are affected while others are not. Once the meaning of this heterogeneity is discovered, it can be used to devise a cocktail of therapeutics that will treat the whole tumor. Such non-invasive tests are more efficient than biopsies, because one can never be sure of an accurate sampling of the heterogeneity of the tumor with a biopsy.

References

[1] A. Jasanoff and P.Z. Sun, *In vivo* magnetic resonance microscopy of brain structure in unanesthetized flies, *J Magn Reson* **158**(1–2) (2002), 79–85.

[2] Y. Wang, N.J. Wright, H. Guo, Z. Xie, K. Svoboda, R. Malinow, D.P. Smith and Y. Zhong, Genetic manipulation of the odor-evoked distributed neural activity in the Drosophila mushroom body, *Neuron* **29**(1) (2001), 267–276.

[3] B.M. Putzer, T. Stiewe, F. Rodicker, O. Schildgen, S. Ruhm, O. Dirsch, M. Fiedler, U. Damen, B. Tennant, C. Scherer, F.L. Graham and M. Roggendorf, Large nontransplanted hepatocellular carcinoma in woodchucks: treatment with adenovirus-mediated delivery of interleukin 12/B7.1 genes, *J Natl Cancer Inst* **21;93**(6) (March 2001), 472–479.

[4] J.J. Galvez, R.J. Munn, A.S. Garbott and R.D. Cardiff, The development of the MMHCC image archives, UCD Center for Comparative Medicine, University of California, Davis, 95616, In press, (http://www.pathology.pitt.edu/apiii02/Sci-Galvez.htm.)

[5] M.J. Paulus, S.S. Gleason, M.E. Easterly and C.J. Foltz, A review of high-resolution X-ray computed tomography and other imaging modalities for small animal research, *Lab Anim* (*NY*) **30**(3) (2001), 36–45.

[6] S.J. Kennel, I.A. Davis, J. Branning, H. Pan, G.W. Kabalka and M.J. Paulus, High resolution computed tomography and MRI for monitoring lung tumor growth in mice undergoing radioimmunotherapy: correlation with histology, *Med Phys* **27**(5) (2000), 1101–1107.

[7] D. Lapointe, N. Brasseur, J. Cadorette, C. La Madeleine, S. Rodrigue, J.E. van Lier and R. Lecomte, High-resolution PET

imaging for *in vivo* monitoring of tumor response after photodynamic therapy in mice, *J Nucl Med* **40**(5) (1999), 876–882.
[8] C. Bremer, C.H. Tung and R. Weissleder, *In vivo* molecular target assessment of matrix metalloproteinase inhibition, *Nat Med* **7** (2001), 743–748.
[9] C. Bremer, S. Bredow, U. Mahmood, R. Weissleder and C.H. Tung, Optical imaging of matrix metalloproteinase-2 activity in tumors: feasibility study in a mouse model, *Radiology* **221**(2) (Nov 2001), 523–529.
[10] L.D. Stegman, A. Rehemtulla, D.A. Hamstra, D.J. Rice, S.J. Jonas, K.L. Stout, T.L. Chenevert and B.D. Ross, Diffusion MRI detects early events in the response of glioma model to the yeast cytosine deaminase gene therapy strategy, *Gene Ther* **7** (2000), 1005–1010.
[11] K. Turetschek, E. Floyd, T. Helbich, T.P. Roberts, D.M. Shames, M.F. Wendland, W.O. Carter and R.C. Brasch, MRI assessment of microvascular characteristics in experimental breast tumors using a new blood pool contrast agent (MS-325) with correlations to histopathology, *J Magn Reson Imaging* **14**(3) (2001), 237–242.
[12] G.H. Patterson and J. Lippincott-Schwartz, A photoactivatable GFP for selective photolabeling of proteins and cells, *Science* **297** (2002), 1973–1977.
[13] R.E. Campbell, O. Tour, A.E. Palmer, P.A. Steinbach, G.S. Baird, D.A. Zacharias and R.Y. Tsien, A monomeric red fluorescent protein, *Proc Natl Acad Sci USA* **99** (2002), 7877–7882.
[14] M. Knop, F. Barr, C.G. Riedel, T. Heckel and C. Reichel, Improved version of the red fluorescent protein (drFP583/DsRed/RFP), *Biotechniques* **33** (2002), 592, 594, 596–598.
[15] H. Aizawa, M. Sameshima and I. Yahara, A green fluorescent protein-actin fusion protein dominantly inhibits cytokinesis, cell spreading, and locomotion in Dictyostelium, *Cell Struct Funct* **22** (1997), 335–345.
[16] A.D. Izzo, M.A. Machanos, J.T. Beckham and E.D. Jansen, *In vivo* optical imaging of expression of vascular endothelial growth factor following laser incision in skin, *Lasers Surg Med* **29** (2001), 343–350.
[17] K. Doi, J. Hargitai, J. Kong, S.H. Tsang, M. Wheatley, S. Chang, S. Goff and P. Gouras, Lentiviral transduction of green fluorescent protein in retinal epithelium: evidence of rejection, *Vision Res* **42** (2002), 551–558.
[18] F.E. Diehn, N.G. Costouros, M.S. Miller, A.L. Feldman, H.R. Alexander, K.C. Li and S.K. Libutti, Noninvasive fluorescent imaging reliably estimates biomass *in vivo*, *Biotechniques* **33**(6) (Dec 2002), 1250–1252, 1254–1255.
[19] M. Yang, E. Baranov, J.W. Wang, P. Jiang, X. Wang, F.X. Sun, M. Bouvet, A.R. Moossa, S. Penman and R.M. Hoffman, Direct external imaging of nascent cancer, tumor progression, angiogenesis, and metastasis on internal organs in the fluorescent orthotopic model, *Proc Natl Acad Sci USA* **99**(6) (2002), 3824–3829.
[20] R.M. Hoffman, Green fluorescent protein imaging of tumour growth, metastasis and angiogenesis in mouse models, *Lancet Oncology* **3** (2002), 546–556.
[21] C.H. Contag, S.D. Spilman, P.R. Contag, M. Oshiro, B. Eames, P. Dennery, D.K. Stevenson and D.A. Benaron, Visualizing gene expression in living animals using a bioluminiescent reporter, *Potochem Photobiol* **66** (1997), 523–531.
[22] W. Zhang, J.Q.Feng, S.E. Harris, P.R. Contag, D.K. Stevenson and C.H. Contag, Rapid *in vivo* functional analysis of transgenes in mice using whole body imaging of luciferase expression, *Transgenic Res* **10** (2001), 423–434.
[23] F.E. Diehn, N.G. Costouros, M.S. Miller, K.C.P. Li and S.K. Libutti et al., Validation of a small animal noninvasive imaging system for detection of bioluminescence and fluorescence *in vivo* (abs), *Mol Imag* **1** (2002), 200.
[24] B. Laxman, D.E. Hall, M.S. Bhojani, D.A. Hamstra, T.L. Chenevert, B.D. Ross and A. Rehemtulla, Noninvasive real-time imaging of apoptosis, *Proc Natl Acad Sci USA* **99**(26) (2002), 16551–16555.
[25] A. Moore, L. Josephson, R.M. Bhorade, J.P. Basilion and R. Weissleder, Human transferrin receptor gene as a marker gene for MR imaging, *Radiology* **221** (2001), 244–250.
[26] D. Hogemann, L. Josephson, R. Weissleder and J.P. Basilion, Improvement of MRI probes to allow efficient detection of gene expression, *Bioconj Chem* **11** (2000), 941–946.
[27] W. Warren, P. Tian and D. Keusters, Two-photon imaging without fluorescence detection (abs), *Mol Imag* **1** (2002), 242.
[28] A. Moore, S. Bonner-Weir and R. Weissleder, Noninvasive *in vivo* measurement of beta-cell mass in mouse model of diabetes, *Diabetes* **50** (2001), 2231–2236.
[29] J.G. Tjuvajev, G. Stockhammer, R. Desai, H. Uehara, K. Watanabe, B. Gansbacher and R.G. Blasberg, Imaging the expression of transfected genes *in vivo*, *Cancer Res* **55**(24) (1995), 6126–6132.
[30] M. Doubrovin, V. Ponomarev, T. Beresten, J. Balatoni, W. Bornmann, R. Finn, J. Humm, S. Larson, M. Sadelain, R. Blasberg and J. Gelovani Tjuvajev, Imaging transcriptional regulation of p53-dependent genes with positron emission tomography *in vivo*, *Proc Natl Acad Sci USA* **98**(16) (2001), 9300–9305.
[31] C.H. Tung, S. Bredow, U. Mahmood and R. Weissleder, Preparation of a cathepsin D sensitive near-infrared fluorescence probe for imaging, *Bioconjug Chem* **10**(5) (1999), 892–896.
[32] W.H. Li, G. Parigi, M. Fragai, C. Luchinat and T.J. Meade, Mechanistic studies of a calcium-dependent MRI contrast agent, *Inorg Chem* **41**(15) (2002), 4018–4024.
[33] N. Raghunand, C. Howison, A.D. Sherry, S. Zhang and R.J. Gillies, Renal and systemic pH imaging by contrast-enhanced MRI, *Magn Reson Med* **49**(2) (Feb 2003), 249–257.