Minireview

Molecular Imaging and Cancer

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Abstract

Molecular imaging is a relatively new discipline, which developed over the past decade, initially driven by *in situ* reporter imaging technology. Noninvasive *in vivo* molecular imaging developed more recently and is based on magnetic resonance and nuclear (positron emission tomography; gamma camera) and *in vivo* optical imaging systems. Molecular imaging has its roots in both molecular biology and cell biology, as well as imaging technology. Most current *in vivo* molecular imaging strategies are "indirect" and involve the coupling of a "reporter/marker gene" with a complimentary "reporter/marker probe." Imaging the level of probe accumulation provides indirect information related to the level of reporter gene expression. Reporter gene constructs are driven by upstream promoter/enhancer elements that function as an "on/off switch"; they can be constitutive, leading to continuous transcription to identify the site and monitor the level and duration of gene (vector) activity. Alternatively, they can be inducible, leading to controlled gene expression, or they can function as a sensor element to monitor the level of endogenous promoters and transcription factors. Three indirect strategies for imaging therapeutic transgenes are discussed. Several examples of imaging endogenous biological processes in animals using reporter constructs, radiolabeled probes, and positron emission tomography imaging are reviewed (p53-dependent gene expression, T-cell receptor-dependent activation of T-lymphocytes, and trafficking of T-lymphocytes). Issues related to the translation of noninvasive molecular imaging technology into the clinic are discussed.

Introduction

The extraordinary developments in both molecular/cellular biology and noninvasive imaging over the past 2 decades occurred largely in parallel with little direct interaction. However, this began to change less than a decade ago when "reporter" gene technology was first applied to *in situ* imaging of tissue sections (1–3) and applied later to noninvasive, *in vivo* imaging. Three different noninvasive imaging technologies developed more or less in parallel: (*a*) MR[2] imaging (4–8); (*b*) nuclear imaging (quantitative autoradiography, gamma camera and PET; Refs. 9–13); and (*c*) *in vivo* optical imaging of small animals (14–16). These developments led to the term "molecular imaging," which was coined in the mid 1990s. This new field of investigation has expanded rapidly, particularly after several NCI-sponsored initiatives. "Cancer imaging" was identified as one of six "extraordinary scientific opportunities" by NCI in 1997–1998. Subsequent funding initiatives have provided a major stimulus to further the development of this new discipline. Substantial resources have been made available to the research community through NCI's Small Animal Imaging Resources Program and the In Vivo Cellular and Molecular Imaging Centers program. Similar funding initiatives have been developed by other NIH Institutes and by the Department of Energy. In addition, a new NIH institute, the Institute for Biomedical Imaging and Engineering, has recently been formed to better represent the breadth of an expanding imaging community.

Molecular imaging has its roots in both molecular and cell biology as well as in imaging technology. These disciplines have now converged to provide a well-established foundation for exciting new research opportunities and for translation into clinical applications, *e.g.,* established *ex vivo* molecular assays require invasive sampling procedures that preclude sequential studies in the same animal or in human subjects. Tissue sampling may not always adequately represent the biochemical or pathological process under investigation because of tissue heterogeneity, which is especially characteristic of some tumors. Furthermore, temporal studies that use molecular biological assays have required large numbers of animals that are sacrificed at specific time points to achieve a statistically significant temporal profile. The development of sensitive imaging-based assays to monitor molecular genetic and cellular processes *in vivo* would be of considerable value in the study of animal models of human disease, including transgenic animals, as well as for studies in human subjects. Noninvasive imaging of molecular genetic and cellular processes will compliment established *ex vivo* molecular biological assays, and imaging can provide a spatial as well as temporal dimension to our understanding of various diseases.

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[2] The abbreviations used are: MR, magnetic resonance; PET, positron emission tomography; HSV1-tk, herpes simplex virus type 1 thymidine kinase; NCI, National Cancer Institute; IRES, internal ribosomal entry site; FHBG, 9-[4-fluoro-3-(hydoxymethyl)butyl]guanine; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; TK, thymidine kinase; GFP, green fluorescent protein; FIAU, 5-iodo-2′-fluoro-2′deoxy-1-β-D-arabino-furanosyl-uracil; GCV, ganciclovir; RASON, radiolabeled oligonucleotide; egfp, enhanced green fluorescent protein; hSSTR2, human somatostatin receptor subtype-2; NFAT, nuclear factor of activated lymphocyte; TCR, T-cell receptor; hD2R, human dopamine 2 receptor.

Recent progress in our understanding of the molecular genetic mechanisms of many diseases and the application of new biologically based approaches in therapy are exciting new developments. New gene-based therapies can provide control over the level, timing, and duration of action of many biologically active transgene products by including specific promoter/activator regulatory elements in the genetic material transferred. Noninvasive imaging of molecular genetic and cellular processes will accelerate these developments and lead to more effective therapeutic strategies. Methods are being developed for: (*a*) controlled gene delivery to various somatic tissues and tumors using novel gene constructs; (*b*) targeting vectors to specific tissues/organs; and (*c*) controlling gene expression using cell-specific, replication-activated, and drug-controlled expression systems (17–19). A noninvasive, clinically applicable method for quantitatively imaging the expression of transduced genes in target tissue or specific organs would be of considerable value. It will facilitate the monitoring and evaluation of gene therapy in human subjects by defining the location(s), magnitude, and persistence of gene expression over time.

Imaging Strategies

Two imaging strategies, "direct" and "indirect," will be discussed, and several examples will be provided. "Direct imaging" strategies are based on imaging the target directly, usually with a target-specific probe, and can be defined in terms of a probe–target interaction. The resultant image of probe localization and magnitude (image intensity) is directly related to its interaction with the target epitope or enzyme. Indirect molecular imaging is a little more complex in that it may involve multiple components. One example of indirect imaging that is now being widely used is "reporter imaging," which usually includes a "reporter gene" and "probe." The reporter gene product can be an enzyme that converts a reporter probe to a metabolite that is selectively trapped within transduced cells. Alternatively, the reporter gene product can be a receptor or transporter that "irreversibly traps" the probe in transduced cells during the period of image acquisition. Indirect imaging paradigms are currently more widely used in molecular imaging and will be discussed in greater detail below.

Direct imaging strategies are common in nuclear medicine and include monoclonal antibody targeting of a particular cell membrane epitope, imaging the activity of a particular enzyme (*e.g.,* hexokinase) with an enzyme-specific probe (*e.g.,* deoxyglucose), or imaging the activity of a particular transporter with a transporter-specific probe. Imaging cell surface-specific antigens or epitopes with radiolabeled antibodies, minibodies, or peptides is an example of direct molecular imaging that has developed over the past 30 years. PET imaging of receptor density/occupancy using small radiolabeled molecular probes has also been widely used, particularly in neuroscience research. These examples represent some of the first "molecular imaging" applications used in clinical nuclear medicine research.

A more recent direct imaging strategy involves the development of antisense and aptomer oligonucleotide probes that specifically hybridize to target mRNA or proteins *in vivo*. Radiolabeled antisense probes are RASONs that have been developed to directly image endogenous gene expression at the transcriptional level. RASONs are small oligonucleotide sequences that are complimentary to a small segment of target mRNA or DNA and could potentially target any specific mRNA or DNA sequence. In this context, imaging specific mRNAs with RASONs produces direct images of specific molecular genetic events. Some efficacy for gamma camera and PET imaging endogenous gene expression using RASONs has been reported (20–23). Nevertheless, RASON imaging has several serious limitations, including: (*a*) low number of target mRNA/DNA molecules per cell; (*b*) limited tracer delivery (poor cell membrane and vascular permeability, cannot penetrate blood–brain barrier); (*c*) poor stability (degradation by H-RNase); (*d*) slow clearance (slow washout of nonbound oligonucleotides); and (*e*) comparatively high background activity and low specificity of localization (low target/background ratios). Imaging specific RASON targets in the body is complicated and interpretation of the images must be approached with caution.

Indirect molecular imaging is currently the most widely used strategy for radionuclide-based imaging (24, 25), as well as for optical (14–16) and MR (7, 8) imaging. Most indirect molecular imaging paradigms involve the use of reporter transgene technology and specific probes to produce an image that reflects reporter gene expression. Although reporter gene imaging initially used optical technology that frequently required postmortem tissue sampling and processing (*e.g.,* β-galactosidase assay), more recent studies have emphasized noninvasive imaging techniques involving live animals and human subjects. This noninvasive approach involves a reporter transgene (*e.g., HSV1-tk*) placed under the control of upstream promoter/enhancer elements. These promoter/enhancer elements can "always be turned on" with constitutive promoters (*e.g.,* long terminal repeat, Rous sarcoma virus, and cytomegalovirus), or they can be "sensitive" to activation by specific endogenous transcription factors (factors that bind to and activate specific enhancer elements). Several noninvasive imaging paradigms have been described, and it has recently been shown that transcriptional regulation of endogenous (host tissue) gene expression can be imaged using both nuclear (PET) and optical (fluorescence) imaging (26, 27).

Reporter Gene Imaging

A common feature of all reporter vectors is the cDNA expression cassette containing the reporter transgene(s) of interest (*e.g.,* HSV1-*tk*). The advantage and versatility of reporter vectors is that the design and arrangement of the expression cassette can be varied (28), *e.g.,* the reporter transgene(s) can be driven by any promoter/enhancer sequence of choice. The promoter can be "constitutive," leading to continuous transcription, or it can be inducible, leading to controlled expression. The promoter can also be cell specific, allowing expression of the transgene to be restricted to certain cells and organs. The paradigm for quantitative imaging of transgene expression involves several steps, including the initiation of transcription (that can be controlled by specific promoter/enhancer elements),

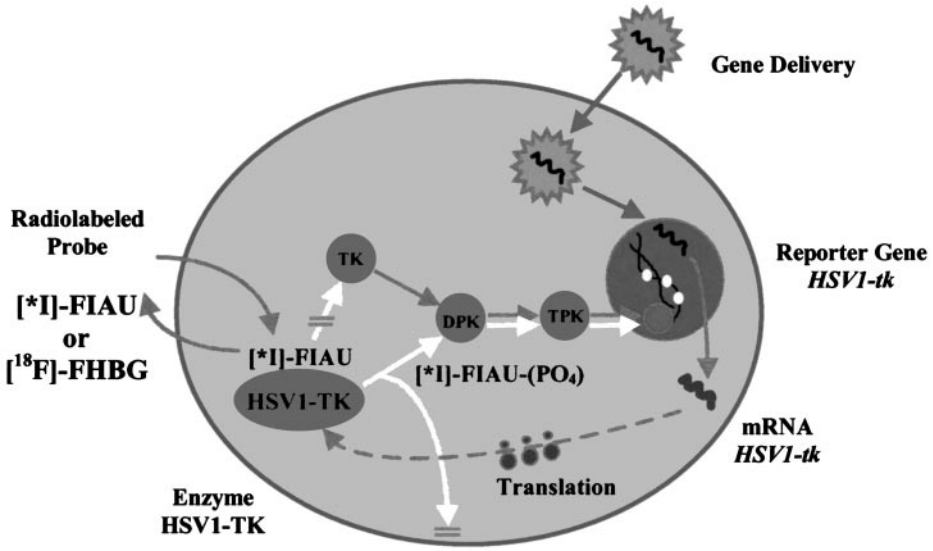

Fig. 1. Schematic for imaging HSV1-tk marker/reporter gene expression with marker/reporter probes FIAU and FHBG. The *HSV1-tk* gene complex is transfected into target cells by a vector (retrovirus, adenovirus, liposome, or any other vector). Inside the transfected cell, the *HSV1-tk* gene is transcribed to *HSV1-tk* mRNA and then translated on the ribosomes to a protein (enzyme), HSV1-TK. After administration of a complimentary radiolabeled marker/reporter probe (*FIAU* or *FHBG*) and transport into the cell, the probe is phosphorylated by HSV1-TK (gene product). The phosphorylated radiolabeled marker/reporter probe does not readily cross the cell membrane; it is "trapped" and accumulates within the cell. Thus, the magnitude of marker/reporter probe accumulation reflects the level of HSV1-TK enzyme activity and the level of *HSV1-tk* gene expression.

the process of DNA transcription and stabilization of mRNA, and subsequent translation of mRNA into the gene product (a protein). In this manner, the reporter expression cassette can be designed to provide information about endogenous gene regulation, mRNA stabilization, and specific protein–protein interactions.

A general paradigm for noninvasive reporter gene imaging using radiolabeled probes was initially described in 1995 (9) and is diagrammatically shown in Fig. 1. This paradigm requires the appropriate combination of a reporter/marker transgene and probe. It is important to note that imaging transgene expression is independent of the vector used to transfect/transduce target tissue; namely, any of several currently available vectors can be used (*e.g.,* retrovirus, adenovirus, adeno-associated virus, lentivirus, liposomes, etc.). The reporter transgene usually encodes for an enzyme (*e.g.,* HSV1-TK) that selectively metabolizes the radiolabeled probe and results in its entrapment and accumulation in the transduced cell. It may be helpful to consider this reporter imaging paradigm as an example of an *in vivo* enzymatic radiotracer assay that reflects reporter gene expression. Enzymatic amplification of the signal (*e.g.,* level of radioactivity) facilitates imaging the location and magnitude of reporter gene expression. Viewed from this perspective, reporter gene imaging is similar to imaging hexokinase activity with fluorodeoxyglucose.

Wild-type *HSV1-tk* (11) and a mutant *HSV1-tk* gene, *HSV1-sr39tk* (29), are the reporter genes most commonly used in current molecular imaging studies using radiolabeled probes and PET imaging. The *HSV1-tk* and *HSV1-sr39tk* gene products are proteins (enzymes) that have less substrate specificity than mammalian TK1. The viral kinases phosphorylate a wider range of compounds, including acycloguanosines (*e.g.,* acyclovir, GCV, and FHBG) and 2′-fluoro-nucleoside analogues of thymidine (*e.g.,* FIAU). This difference between mammalian and viral TK enzymes permits the development and use of radiolabeled probes that are phosphorylated to a significantly greater extent by HSV1-TK or HSV1-sr39TK in comparison with mammalian TK1.

Alternatively, a reporter gene can encode for an extracellular or intracellular receptor that "irreversibly" binds or transports a radiolabeled or paramagnetic probe. *hD2R* is an example of such a reporter gene (30). This was a very clever strategy because hD2R expression is largely limited to the striatal–nigral system of the brain and because an established radiolabeled probe, 3-(2′-[^{18}F]fluoroethyl) spiperone, has been extensively used to image striatal–nigral D2 receptors in human subjects (31). Similarly, the *hSSTR2* gene has been suggested as a potential reporter gene for human studies (32, 33), because hSSTR2 expression is largely limited to carcinoid tumors. There is also a complimentary radiolabeled somatostatin analogue ([^{111}In]diethylenetriaminepentaacetic acid-octreotide) that can be used for imaging hSSTR2 expression (34). Radiolabeled octreotide has also been approved for administration to patients with carcinoid tumors. Both of these reporter systems have distinct benefits with respect to initiating molecular/reporter imaging in human subjects. However, receptor expression on the surface of cells is a complex process and involves intracellular trafficking and cell membrane expression that is likely to be altered under different conditions and disease states. It remains to be shown whether imaging receptor-based reporter systems (*e.g.,* the *hD2R* and *hSSTR2* reporter gene systems) will provide a consistent and reliable measure of reporter gene expression. In either case, the level of probe accumulation (level of radioactivity) must be shown to be proportional to the level of gene expression.

Imaging the Expression of Therapeutic Transgenes

A noninvasive, clinically applicable method for imaging the expression of successful gene transduction in target tissue or specific organs of the body would be of considerable value. It would facilitate the monitoring and evaluation of

gene therapy in human subjects by defining the location, magnitude, and persistence of gene expression over time. Targeting gene therapy to particular tissue (*e.g.,* tumor) or specific organs is an increasingly active area of research with 519 related articles published in 1991, 1424 articles in 1995, and 4165 articles in 2000 based on a MEDLINE search.

Several issues that are important for clinical optimization of gene therapy remain unresolved in many current clinical protocols: (*a*) Has gene transduction or transfection been successful?; (*b*) Is the distribution of the transduced or transfected gene localized to the target organ or tissue, and is the distribution in the target optimal?; (*c*) Is the level of transgene expression in the target organ or tissue sufficient to result in a therapeutic effect?; (*d*) Does the transduced or transfected gene localize to any organ or tissue at sufficient levels to induce unwanted toxicity?; (*e*) In the case of combined pro-drug gene therapy protocols, when is transgene expression maximum (optimal), and when is the optimal time to initiate treatment with the pro-drug?; and (*f*) How long does transgene expression persist in the target and other tissues?

We and others have proposed that noninvasive imaging techniques using selected reporter gene and reporter probe combinations will provide a practical and clinically useful way to identify successful gene transduction and expression in patients undergoing gene therapy. One could argue that biopsies of target tissue could be performed and that imaging is not critical. However, imaging provides some clear advantages, including: (*a*) the ability to repeatedly assess gene expression over time, especially when multiple sequential biopsies are not feasible; (*b*) the absence of any perturbation of the underlying tissue that occurs with biopsy procedures; and (*c*) the ability to obtain spatial information in the entire body, as well as target organs and tumors, which could be of considerable value when addressing toxicity issues.

HSV1-*tk* has the advantage of being both a "therapeutic gene" (combined with GCV treatment) and a reporter gene (using an appropriate radiolabeled probe, such as FIAU or FHBG). Experimental validation of this approach has been demonstrated in animal models of colorectal metastases to the liver treated with adenoviral-mediated *HSV1-tk* gene transfer and GCV ("suicide" gene therapy; Refs. 35 and 36) or treatment with conditionally replicating, oncolytic herpes viruses that constitutively express the *HSV1-tk* gene (37, 38). However, most therapeutic genes do not lend themselves to direct imaging of their transgene product. Furthermore, the development and validation of a "new" probe and imaging paradigm specific to each therapeutic transgene of interest would be a very costly and time-consuming endeavor. Many therapeutic gene products do not lend themselves to radionuclide, paramagnetic, or optical assessments. It is therefore reasonable to consider alternative strategies for indirect imaging of therapeutic gene expression; strategies that use established reporter genes and their complimentary radiolabeled probes to provide images that define the location, magnitude, and duration of therapeutic gene expression.

Several indirect reporter gene imaging strategies have been described that can be used to image many different therapeutic genes. These strategies take advantage of using established reporter gene–reporter probe combinations and achieve the objective of monitoring therapeutic gene expression. One strategy uses a fusion gene containing cDNA from both the reporter and therapeutic genes (39). A second strategy uses a *cis*–linked reporter gene (24, 25); a third strategy uses multiple vectors, where one or more vectors carry a therapeutic gene, and another vector carries the reporter gene (40). All three strategies are based on demonstrating a proportional and constant relationship in the coexpression of two or more transgenes over many expression levels.

Strict coexpression of two proteins in equimolar amounts can only be achieved by a fusion gene construct that encodes the cDNA sequence of both genes. This approach is based on existing fusion gene technology, where two genes (one "therapeutic" and one "reporter") are "linked" in a fixed and definable manner. Transcription of the fusion gene occurs under the control of definable upstream promoter/enhancer elements, and translation proceeds to yield a single "fusion protein" (gene product). The fusion protein is a single hybrid of two individual proteins; it is formed from the amino acid sequences encoded in both the therapeutic and reporter genes. Thus, information obtained by imaging the "reporter" component will provide corresponding information about the "therapeutic" component of the fusion protein (39).

There are a number of potential disadvantages of the fusion gene approach: (*a*) the fusion construct may not produce in a functional gene product; this could be attributable to a change in the conformational structure of the native protein or result in an alteration in the subcellular localization (intracellular trafficking) of the fusion protein, or it could reflect a loss of binding or enzymatic activity of its "therapeutic component," as well as to a loss of activity of its "reporter component"; (*b*) transcription modulation of the fusion mRNA or a change in the clearance (breakdown) of the fusion protein may be sufficiently different compared with the two native proteins (gene products of the two native genes); such differences could have a significant impact on the level of the fusion gene product and, thereby, on the level of its biological activity; and (*c*) fusion proteins are larger than the two corresponding native proteins and more likely to generate an immunological response *in vivo*; thus, fusion gene technology cannot be generalized and may not be widely applicable in clinical imaging of therapeutic gene expression. However, when the fusion gene product is functional and nonimmunogenic, it provides a very useful approach to monitor therapeutic gene expression.

A second approach that was recently described involves the proportional expression of two *cis*-linked genes, using an IRES element within a single bicistronic transcription cassette (24, 25). The IRES element enables translation initiation within the bicistronic mRNA, thus permitting gene coexpression by cap-dependent translation of the first cistron and cap-independent, IRES-mediated translation of the second cistron (33, 41, 42). Imaging studies in animals have suggested that proportional coexpression is reliable and quantitative. However, it will be important to demonstrate that IRES-based transgene coexpression remains proportional in different tissues (*e.g.,* that the half-life of each encoded protein remains proportional in different tissues). This is neces-

sary when noninvasive imaging is used to assess organ (tissue) specificity, as well as the level and duration of therapeutic transgene expression.

A third approach for achieving proportional coexpression of one or more therapeutic genes and a reporter gene is based on the administration of multiple vectors. In this paradigm, each vector is similar and expresses a single transgene, a therapeutic or reporter gene. Each vector would have identical envelope characteristics and identical promoter/enhancer elements driving the expression of the therapeutic and reporter transgenes. An infusion cocktail containing the vectors in definable (known) combinations can be administered. A key requirement for this approach is that: (*a*) the multiple vectors transfect and transduce target organs and tissue proportionate to their ratio in the administered cocktail; (*b*) the therapeutic and reporter genes are coexpressed proportionally in all target organs and tissue; and (*c*) the proportionality of coexpression is constant over many expression levels. A recent report has provided encouraging results in support of using multiple vectors (adenovirus) to deliver reporter and therapeutic genes to target organs (40).

Imaging Endogenous Biological Processes

Reporter gene imaging is being used to visualize transcriptional and post-transcriptional regulation of target gene expression, as well as specific intracellular protein–protein interactions. Several examples will be provided below.

Imaging transcriptional regulation of endogenous genes in living animals (and potentially in human subjects) using noninvasive imaging techniques is providing a clearer understanding of normal and cancer-related biological processes. A recent study from our group (26) was the first to show that p53-dependent gene expression can be imaged *in vivo* with PET and by *in situ* fluorescence. A retroviral vector (*Cis*-p53/*TKeGFP*) was generated by placing the herpes simplex virus type 1 TK and e*gfp* fusion gene (*TKeGFP*, a dual-reporter gene) under control of a p53-specific response element. DNA damage-induced up-regulation of p53 transcriptional activity was demonstrated and correlated with the expression of p53-dependent downstream genes, including p21. These findings were observed in U87 (p53 +/+) cells and xenografts but not in SaOS (p53 −/−) cells. This was the first demonstration that a *Cis*-reporter system (*Cis*-p53/TKGFP) was sufficiently sensitive to image endogenous gene expression using noninvasive nuclear (PET) imaging (Fig. 2*a*). The PET images corresponded with up-regulation of genes in the p53 signal transduction pathway (p53-depndent downstream genes) in response to DNA damage induced by BCNU chemotherapy (Fig. 2*b*). PET imaging of p53 transcriptional activity in tumors using the *Cis*-p53TKGFP reporter system could be used to assess the effects of new drugs or other novel therapeutic paradigms that are mediated through p53-dependent pathways, *e.g.,* specific p53 gene therapy strategies that are based on p53 overexpression (43) could be monitored by noninvasive imaging.

It should also be pointed out that the dual reporter construct (*TKeGFP,* fusion gene) provides the opportunity for multimodality (both nuclear and optical imaging) imaging of endogenous gene expression *in vivo*. The *TKeGFP* reporter gene could be introduced into other reporter assay systems to assess other molecular biological pathways. It should also be possible to use the *TKeGFP* reporter gene in transgenic animals; this will facilitate the monitoring and assessment of newly cloned genes or novel signal transduction pathways. Another advantage of the dual reporter system is the ability to compare the images of reporter gene expression obtained with PET, gamma camera, or autoradiography with corresponding *in situ* GFP fluorescence images. The comparison between GFP fluorescence and autoradiographic images, coupled with histology of corresponding tissue sections, provides for spatial and quantitative assessments of reporter gene expression at the microscopic, as well as macroscopic, level.

T-cell activation is an essential component of the immune response in many normal and disease states. The objective of a recent study in our laboratory (27) was to monitor and assess TCR-dependent activation *in vivo* using noninvasive PET imaging. A retroviral vector (*Cis*-NFAT/TKeGFP) was generated by placing the fusion gene (*TKeGFP*) under control of the NFAT response element. A human T-cell leukemia cell line (Jurkat) that expresses a functional TCR was transduced with the *Cis*-NFAT/TKGFP reporter vector and used in these studies. Known activators of T cells (anti-CD3 and -CD28 antibody) produced significantly higher levels of *TKGFP* reporter gene expression (increased GFP fluorescence, levels of HSV1-tk mRNA, and [^{14}C]FIAU accumulation *in vitro*) in *Cis*-NFAT/TKGFP+ Jurkat cells, in comparison with nontreated or nontransduced cells. In mice with focal *Cis*-NFAT/TKGFP+ Jurkat cell infiltrates, similar results were observed in the microPET images (Fig. 3*a*) and *in vivo* fluorescence images (Fig. 3*b*). A strong correlation of *TKGFP* coexpression with up-regulation of T-cell activation markers (CD69 and interleukin-2 production) was demonstrated both *in vitro* and *in vivo*. These results demonstrated that: (*a*) activation of the NFAT signal transduction pathway occurs after TCR stimulation and (*b*) PET imaging of T-lymphocyte activation in tumors after TCR engagement is feasible using the described *TKGFP*-based *Cis*-reporter system. This imaging paradigm could be used to assess the efficacy of novel antitumor vaccines and adoptive immunotherapy.

The trafficking and localization of T-lymphocytes are important components of the immune response and in the elimination of abnormal cells and infectious agents. Passive (*ex vivo*) labeling of T cells with radioactive isotopes can be unstable and does not account for proliferation of activated T cells in the body. An objective of ongoing studies in our laboratory is to image the *in vivo* targeting and accumulation of EBV lymphoma-specific CTLs in allogeneic HLA-matched EBV B-lymphomas using PET and optical imaging modalities. CTLs specific for homogenous EBV-transformed B-lymphocytes were obtained and stably transduced with a constitutively expressing dual reporter gene (*HSV1tk/egfp* fusion gene). Specific accumulation and localization of radioactivity were observed only in the autologous EBV(+) lymphoma, the allogeneic HLA-matched EBV(+) lymphoma, and spleen; no localization was seen in the allogeneic HLA-matched non-EBV lymphoma and HLA-mismatched EBV(+) lymphoma. Sequential imaging over 72 h in another set of animals showed

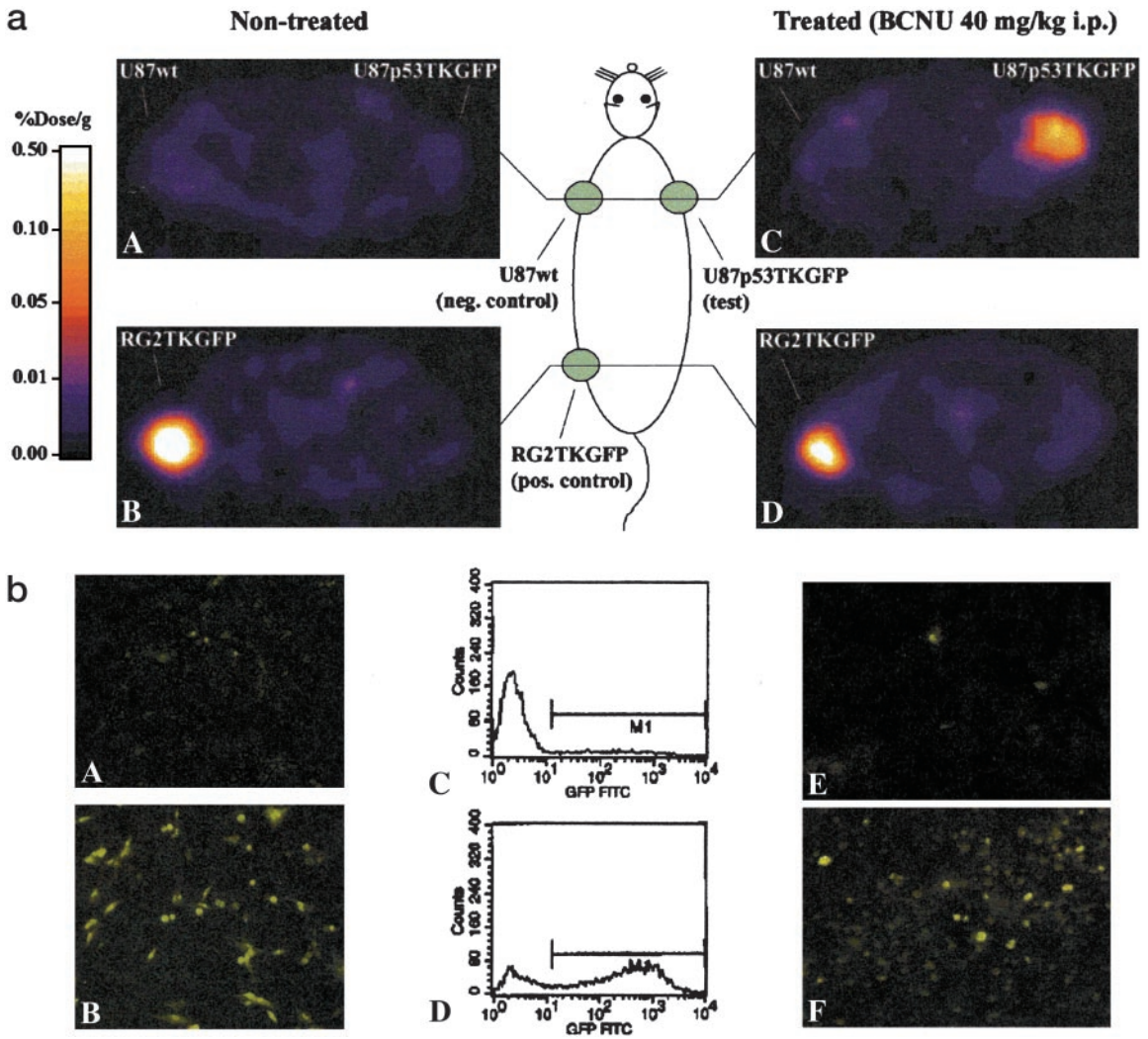

Fig. 2. *a,* PET imaging of endogenous p53 activation (26). Transaxial PET images (GE Advance tomograph) through the shoulder (*A* and *C*) and pelvis (*B* and *D*) of two rats are shown; the images are color coded to the same radioactivity scale (% dose/gram). An untreated animal is shown on the *left,* and a BCNU-treated animal is shown on the *right*. Both animals have three s.c. tumor xenografts: (*a*) U87p53TKGFP (*test*) in the right shoulder; (*b*) U87 wild type (*neg. control*) in the left shoulder; and (*c*) RG2TKGFP (*pos. control*) in the left thigh. The nontreated animal on the *left* shows localization of radioactivity only in the positive control tumor (*RG2TKGFP*); the test (*U87p53TKGFP*) and negative control (*U87wt*) tumors are at background levels. The BCNU-treated animal on the *right* shows significant radioactivity localization in the test tumor (right shoulder) and positive control (left thigh) but no radioactivity above background in the negative control (left shoulder). *b* (26), validation of *Cis*-p53/TKGFP reporter system in cell cultures (*left* and *middle panels*). Fluorescence microscopy and fluorescence-activated cell sorter analysis of a transduced U87p53/TKGFP cell population in the noninduced (control) state (*A* and *C*) and 24 h after a 2-h treatment with 40 μg/ml BCNU (*B* and *D*). Assessment of *Cis*-p53/TKGFP reporter system in U87p53/TKGFP s.c. tumor tissue (*right panel*). Fluorescence microscopy images of U87p53/TKGFP s.c. tumor samples obtained from nontreated rats (*E*) and rats treated with 40 mg/kg BCNU i.p. (*F*).

trafficking, as well as targeting, of the transduced and radiolabeled CTLs. These preliminary studies indicate that it may be feasible to isolate and transduce CTLs (and other immune-specific cells) with reporter constructs and then monitor their targeting and proliferation in the donor or HLA-matched recipient using noninvasive reporter gene PET imaging.

Concluding Comments

The field of molecular imaging has enjoyed exceptional growth over the past 5 years; this is particularly striking because it has been identified as a new discipline in only the past decade. This rapid growth is caused by the established base of three imaging technologies (nuclear, MR, and optical), coupled with established programs in molecular and cell biology. However, it is the convergence of these disciplines that is at the heart of this success story and is the wellspring for additional advances in this new field. Continued success in the future depends on bringing the imaging disciplines closer together (as we have seen in the complimentary benefits of dual-reporter, optical and nuclear, imaging paradigms), as well as further involvement with our molecular and cell biology colleagues.

Complimentary instrumentation for imaging both animals and patients provides a unique opportunity for substantive translational research. The opportunities for novel molecular imaging research in animals are very bright indeed. Animals

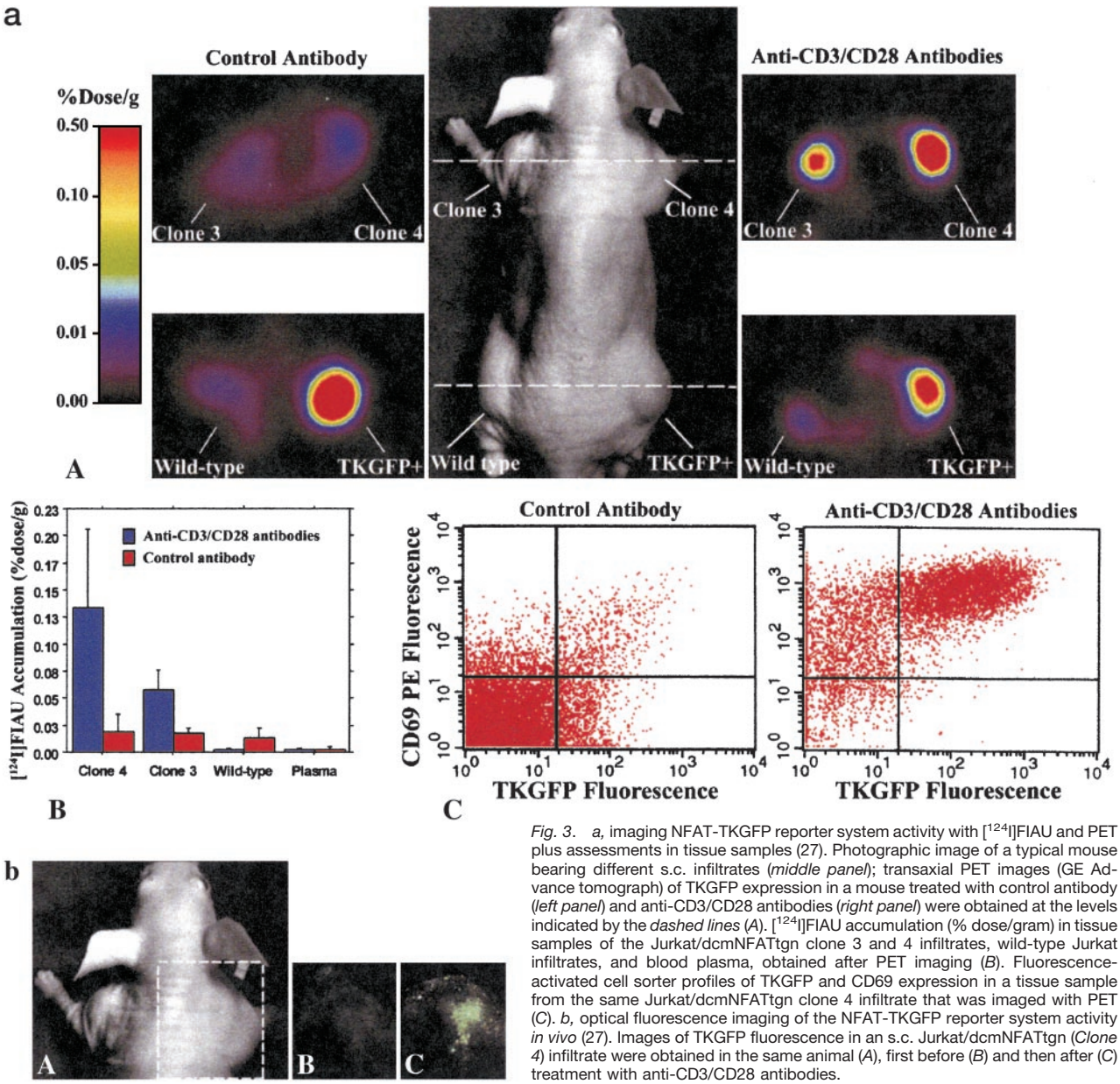

Fig. 3. *a,* imaging NFAT-TKGFP reporter system activity with [^{124}I]FIAU and PET plus assessments in tissue samples (27). Photographic image of a typical mouse bearing different s.c. infiltrates (*middle panel*); transaxial PET images (GE Advance tomograph) of TKGFP expression in a mouse treated with control antibody (*left panel*) and anti-CD3/CD28 antibodies (*right panel*) were obtained at the levels indicated by the *dashed lines* (*A*). [^{124}I]FIAU accumulation (% dose/gram) in tissue samples of the Jurkat/dcmNFATtgn clone 3 and 4 infiltrates, wild-type Jurkat infiltrates, and blood plasma, obtained after PET imaging (*B*). Fluorescence-activated cell sorter profiles of TKGFP and CD69 expression in a tissue sample from the same Jurkat/dcmNFATtgn clone 4 infiltrate that was imaged with PET (*C*). *b,* optical fluorescence imaging of the NFAT-TKGFP reporter system activity *in vivo* (27). Images of TKGFP fluorescence in an s.c. Jurkat/dcmNFATtgn (*Clone 4*) infiltrate were obtained in the same animal (*A*), first before (*B*) and then after (*C*) treatment with anti-CD3/CD28 antibodies.

bearing transduced xenografts containing specific reporter constructs and animals transduced with viral vectors bearing similar reporter constructs currently provide the main resource for *in vivo* molecular imaging research. Transgenic animals expressing reporter constructs system wide or reporter constructs that are expressed only in certain organs or cell types have been developed for optical imaging studies. Similar transgenic animals are being developed for nuclear and MR imaging with radiolabeled and paramagnetic probes. Noninvasive imaging can assess the activation or suppression of specific genes and activity of specific signal transduction pathways. This information could be useful in defining and monitoring cell transformation to a more malignant state or whether a specific drug effectively targets a specific signal transduction pathway. Studies designed to test these hypotheses are now being developed.

Reporter gene imaging will initially be limited in patients because of the necessity of transducing target tissue with specific reporter constructs. Ideal vectors for targeting specific organs or tissue (tumors) do not exist, although this is a very active area of human gene therapy research. Each new vector requires extensive and time-consuming safety testing before Food and Drug Administration approval for human administration. Similarly, Food and Drug Administration approval of new radiolabeled and paramagnetic probes for imaging the expression of new reporter systems will be required. The translation of molecular imaging research into patient studies and clinical application will be measured and

carefully monitored. We remain optimistic; the tools and resources largely exist, and we should be able to perform limited gene imaging studies in the near future. The advantages and benefits of noninvasive imaging to monitor transgene expression in gene therapy protocols are clear. The ability to visualize transcriptional and post-transcriptional regulation of endogenous target gene expression, as well as specific intracellular protein–protein interactions in patients, will provide the opportunity for new experimental venues in patients. Optimistically, they include the potential to image the malignant phenotype of a patient's tumor at a molecular level over time and the potential to image a drug's effect on a specific signal transduction pathway in the tumor.

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