Noninvasive Molecular Neuroimaging Using Reporter Genes: Part II, Experimental, Current, and Future Applications

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SUMMARY: In this second article, we review the various strategies and applications that make use of reporter genes for molecular imaging of the brain in living subjects. These approaches are emerging as valuable tools for monitoring gene expression in diverse applications in laboratory animals, including the study of gene-targeted and trafficking cells, gene therapies, transgenic animals, and more complex molecular interactions within the central nervous system. Further development of more sensitive and selective reporters, combined with improvements in detection technology, will consolidate the position of in vivo reporter gene imaging as a versatile technique for greater understanding of intracellular biologic processes and underlying molecular neuropathology and will potentially establish a future role in the clinical management of patients with neurologic diseases.

Molecular imaging seeks to shed new light on both structure and function by creating images that directly or indirectly reflect specific cellular and molecular events (e.g., gene expression) that can reveal pathways and mechanisms responsible for disease within the context of physiologically authentic and intact living subject environments. We and others have previously reviewed the factors contributing to the emergence of molecular imaging, the particular advantages of these approaches, and the general goals potentially achievable in biomedical research and clinical practice by adopting molecular imaging strategies. One of the subdisciplines in molecular imaging that is least familiar to clinical imaging specialists, and arguably one that holds future promise in neuroimaging, is reporter gene expression imaging. In the first article of this series, we reviewed the basic principles and recent technologic developments in reporter gene expression imaging in living subjects. In this second article, we review examples from the myriad experimental applications currently possible in molecular neuroimaging.

Experimental Applications in Molecular Neuroimaging Using Reporter Genes

Four broad categories of experimental applications for reporter gene imaging in the brain are as follows: gene marking of cells and viruses with reporter genes, imaging of gene therapies, imaging of transgenic animals carrying reporter genes, and imaging of more complex intracellular molecular events such as protein trafficking. Some details of recent examples of these applications are displayed in the Tables, with 1 or 2 representative examples of each application discussed in greater detail below. Of note regarding terminology, “xenografts” result from transplantation of cells or tissues from 1 species to another (e.g., human cells into mice), and “orthotopic transplants” refer to grafting cells into the same body location/or- gan as that from which the cells are derived (e.g., glioma cells delivered into brain).

Imaging Gene-Marked Cells

Gene marking may be used to track the behavior of almost any tissue. It is necessary to transfect cells stably with the imaging marker gene if they and their progeny are to be followed for their entire lifespan within the living subject. However, this assumes that minimal or no promoter attenuation or shutoff takes place. The latter can contribute substantially to a decline in transgene expression despite the constitutive nature of the promoter. In practice, transient transfection of cells suffices if these marked cells are to be imaged in a living subject for no more than approximately 7–10 days, depending on the cells in question and other parameters as well. In principle, gene-marker studies may be used to follow the behavior of almost any cell type in living subjects. In clinical practice, this has been mostly used with hematopoietic cells. However, in molecular imaging research, a variety of cells can be engineered to incorporate reporter genes. Usually, gene marking of cells that are static in 1 location (e.g., subcutaneous tumor xenografts or orthotopic brain tumor implants) is used for first assessment and continued validation of reporter genes and their probes, for refining the technical aspects of molecular imaging signal-intensity detection from the brain, or for studying the behavior of the cells themselves within living subjects. This can be accomplished in 2 ways: ex vivo transfection of the cells in question with a vector containing an imaging cassette, followed by placement of these cells in a living subject or direct in vivo placement, usually via injection of the vectors carrying the reporter gene, as part of the recombinant genome of viruses, into the cells of interest within the body.

There are numerous examples of bioluminescence imaging of cells (especially cancer cells) that are mostly destined to remain static in the brain after ex vivo gene marking with imaging reporters and subsequent placement in living rodents (Table 1). A noteworthy advantage in these cancer models is that they create the opportunity for temporal evaluation of cancer biology in a noninvasive manner. Dynamic studies of xenograft growth and regression, either spontaneously or after therapy, can be performed. The enzymatic emission of light by firefly luciferase (Fluc) is adenosine triphosphate–dependent; therefore, only living metabolically active cells contribute to
the signal intensity. A decrease in signal intensity occurs as cells die.

One of the earliest applications of reporter gene imaging of orthotopic mouse brain xenografts of rat 9L gliosarcoma cells gene-marked with Fluc was conducted by Rehemtulla et al. Intracerebral tumor burden was monitored over time by quantification of light emission and tumor volume by using bioluminescence imaging and MR imaging, respectively. There was excellent correlation \((r = 0.91)\) between detected photons and tumor volume. A quantitative comparison of tumor cell kill, determined from serial MR imaging volume measurements and bioluminescence imaging photon counts following 1,3-bis(2-chloroethyl)-1-nitrosourea treatment, revealed that both imaging techniques yielded statistically similar cell kill values \((P = 0.951)\). These results provide direct validation of bioluminescence imaging as a powerful and quantitative tool for the assessment of antineoplastic therapies in living animals.

In a more recent study, Deroose et al. reported the use of bioluminescence imaging to characterize lentiviral vector-mediated gene transfer into mouse brain. Various features of the imaging signals were characterized including localization (Fig 1), kinetics, resolution, and reproducibility. Although light signal intensity gradually decreased to 20% of initial val-

Table 1: Recent examples of applications in molecular neuroimaging using gene marking (of static cells)

| Type of Cell Marked | Method of Gene Marking | Transplant Site | Animal Model | Imaging Method | Application | Reference |
|---------------------|------------------------|-----------------|--------------|----------------|-------------|-----------|
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of technical aspects of neuroimaging by testing suitability of Fluc reporter for brain imaging; evaluation of antineoplastic chemotherapy | 10         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice, rats   | Fluc BLI       | Evaluation of technical aspects of neuroimaging after lentiviral transduction of various cancer cells | 11         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of technical aspects of neuroimaging using a herpes simplex virus amplicon vector expressing Fluc from an inducible promoter | 12         |
| Cancer cells        | Ex vivo                | Orthotopic, heterotopic | Mice | Fluc BLI       | Evaluation of technical aspects of neuroimaging by comparing level and time course of light signal from 2 different locations | 13         |
| Cancer cells        | Ex vivo                | Orthotopic, heterotopic | Mice | HSV1-tk, PET   | Evaluation of technical aspects of neuroimaging by testing suitability of a \(^{76}\)Br-labeled uracil analog as a probe in brain imaging | 14         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of technical aspects of neuroimaging by correlating tumor growth with Fluc BLI and MR imaging; evaluation of antineoplastic chemotherapy | 15         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of technical aspects of neuroimaging when establishing tumors with varying abilities to disrupt the BBB; evaluation of antineoplastic chemotherapy | 16         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of technical aspects of neuroimaging by testing hyperspectral/multispectral light analysis as a means of 3D localization in BLI | 17         |
| Normal brain        | In vivo                | Orthotopic      | Mice         | GFP, fluorescence | Evaluation of technical aspects of neuroimaging using reflectance fluorescence imaging | 18         |
| Normal brain        | In vivo                | Orthotopic      | Rats         | HSV1-tk, PET   | Evaluation of technical aspects of neuroimaging in diagnosing early herpes simplex encephalitis | 19         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of role of activation of G protein-coupled receptor CXCR4 in growth of intracranial tumors; evaluation of antineoplastic chemotherapy | 20         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of immunotherapy of intracranial tumors | 21         |
| Cancer cells        | Ex vivo                | Orthotopic      | Rats         | Fluc BLI       | Evaluation of photodynamic therapy of intracranial tumors | 22         |
| Cancer cells        | Ex vivo                | Orthotopic      | Mice         | Fluc BLI       | Evaluation of tumor angiogenesis by imaging integrin \(\alpha_v\beta_3\) receptor expression using fluorescence imaging | 23         |

Note:—BLI indicates bioluminescence imaging; GFP, green fluorescent protein; PET, positron-emission tomography; HSV1-tk, herpes simplex virus–thymidine kinase; BBB, blood-brain barrier; \(\alpha_v\beta_3\), a vitronectin receptor on the cell surface.

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ues obtained in the first month, the signal intensity remained constant thereafter for more than 1 year after heterotopic brain xenografting of stably transduced 293T cells (Fig 2), allowing the potential for long-term evaluation of novel therapies for experimental brain disorders.

In vivo imaging of cell trafficking is currently performed in clinical practice (eg, by using indium-111 oxine for single-photon emission tomography [SPECT] imaging of infection and inflammation) and is the objective of many immunologic and oncologic studies. Gene marking has the advantage over simple cell labeling for long-term tracking of cells because the imaging gene is passed on to the cell progeny and the imaging signal intensity is not lost through dilution by egress of the label from the cell. When a gene-marked cell dies or is phagocytosed by immune cells, the imaging signal intensity is also lost, unlike the situation with simple cell labeling in which the imaging signal intensity is not dependent on cell viability and may originate from extracellular space or from within immune scavenger cells.

Table 2 outlines examples of recent reports that feature reporter gene imaging of trafficking virus particles, parasites, cancer cells, and stem cells to the brain. Viral marking is used mostly to study the pathogenesis of viral encephalitis, whereas marking of cancer cells may be used to investigate brain metastases from systemic primary sources (eg, breast cancer). An application gaining rapid acceptance in the laboratory is that of reporter gene imaging of neural stem cells to visualize, quantify, and time their trafficking to gliomas, ischemic brain, and injured spinal cord. Kim et al noninvasively imaged the migratory behavior of Fluc-marked neural progenitor cells to middle cerebral artery infarcts in mice and found that intraventricular delivery of stem cells results in earlier and more efficient infarct seeding. More
recently, the same group showed that marked stem cells survived better in T-cell–deficient nude mice than in immuno-competent animals, indicating that immune status and host immunity can have an influence on stem cell graft survival in the cell therapy of experimental stroke.34

**Imaging of Gene Therapies**

Although various methods of gene therapy have met with limited success, it is probable that eventually many diseases will be successfully treated with the delivery of 1 or more transgenes to target tissue. A concern in applying gene therapy is achievement of controlled and effective delivery of genes to target cells and avoidance of ectopic expression. Molecular imaging of reporters on particular therapeutic genes could be critical in optimizing gene therapy.36 The aim of these approaches is to provide a noninvasive surrogate marker for monitoring gene expression. MR images revealed significant reduction in tumor growth rates associated with yCD/5-fluorocytosine (5FC) gene therapy. Significant increases in mean tumor diffusion values were also observed during treatment with 5FC. Moreover, spatial heterogeneity in tumor diffusion changes were also observed, revealing that diffusion MR imaging could detect regional therapeutic effects due to the nonuniform delivery and/or expression of the therapeutic yCD transgene within the tumor mass. In addition, bioluminescence imaging in the living mice detected *Fluc* expression, which was found to decrease with time during administration of the prodrug, providing a noninvasive surrogate marker for monitoring gene expression. These results demonstrated the efficacy of the yCD/5FC strategy for the treatment of brain tumors and revealed the feasibility of using multitechnique molecular and functional imaging for assessment of gene expression and therapeutic efficacy.

**Imaging of Transgenic Models of Spontaneous Disease**

The mouse is close to an ideal system to model human diseases because its genome can be easily manipulated and its anatomy and physiology are similar to that of humans. Combinatorial genetic engineering strategies to generate disease-prone genetic strains are now possible to produce new alleles by transgenic technology, in which extra deoxyribonucleic acid (DNA) that encodes the gene of interest is inserted heritably into the mouse genome or by knockout/knockin technology, in which specific portions of the mouse genome are targeted for selective alteration. For example, cyclization recombinase (Cre) recombinase is an enzyme used to modify genes and chromosomes. A target region to be deleted in a gene locus can be marked for deletion by signal intensity sequences of locus of *X-over* P1 (loxP) that are identified by Cre. The expression of

**Table 2: Recent examples of applications in molecular neuroimaging using gene marking (of trafficking cells)**

| Type of Cell Marked | Method of Gene Marking | Transplant Site | Animal Model | Imaging Method | Application | Reference |
|---------------------|------------------------|----------------|--------------|---------------|-------------|-----------|
| Viruses             | Ex vivo                | Intravenous, or intranasal | Mice | Fluc BLI | Evaluation of effects of interferons on vaccinia viral spread to the brain | 26 |
| Viruses             | Ex vivo                | Intravenous | Mice | Fluc BLI | Evaluation of factors relating to Sindbis viral spread to the brain | 27 |
| Viruses             | Ex vivo                | Intravenous | Mice | Fluc BLI, Rluc BLI | Evaluation of effects of valacyclovir on HSV-1 viral spread to the brain and eyes | 28 |
| Malaria parasites in RBCs | Ex vivo              | Intravenous | Mice, rats | Fluc BLI | Evaluation of biology of parasite sequestration in cerebral malaria | 29 |
| Cancer cells        | Ex vivo                | Intravenous | Mice | Fluc BLI | Evaluation of breast cancer metastasis to brain tumors | 30 |
| Stem cells          | Ex vivo                | Intravenous | Mice | Fluc BLI | Evaluation of trafficking of stem cells to brain tumors | 31 |
| Stem cells          | Ex vivo                | Orthotopic | Mice | Fluc BLI | Evaluation of trafficking of stem cells to brain tumors | 32 |
| Stem cells          | Ex vivo                | Orthotopic | Mice | Fluc BLI | Evaluation of effect of stem cell-delivered chemotherapy on tumor burden | 33 |
| Stem cells          | Ex vivo                | Orthotopic | Mice | Fluc BLI | Evaluation of trafficking of stem cells to brain infarcts | 34 |
| Stem cells          | Ex vivo                | Orthotopic | Mice | Fluc BLI | Evaluation of trafficking of stem cells to ischemic brain in relation to immune status and host immunity | 35 |
| Stem cells          | Ex vivo                | Orthotopic | Mice | Fluc BLI | Evaluation of trafficking of stem cells to injured spinal cord | 36 |

**Note:**—BLI indicates bioluminescence imaging; RBC, red blood cells; Rluc, Renilla luciferase.
Holland et al have developed an imaging approach to measure the retinoblastoma pathway. More recently, Momota and colleagues used this method effectively for testing cancer prevention and treatment. This approach can monitor the onset, progression, and response to therapy and may be particularly useful for evaluating the effects of drug action, each mouse being its own control.43 To this end, several research groups have used bioluminescence neuroimaging in their assessment of transgenic mice (Table 3).44-54 For example, Lin et al46 have imaged transforming growth factor (TGF)-β signaling in living mice in response to brain injury (Fig 3), and Kadurugamuwa et al51 have developed a method to simultaneously image pneumococcal meningitis and the accompanying neuronal injury (Fig 4). Other examples of transgenic models of spontaneous cancer, in which tumor formation is dependent on defined genetic alterations, provide a powerful tool for evaluating the therapeutic efficacy of pathway-specific antineoplastics. Vooijs et al54 have generated a conditional mouse model for retinoblastoma-dependent sporadic cancer that permits noninvasive monitoring of pituitary tumor development in living mice by bioluminescence imaging of Fluc expression. Bioluminescence imaging of pituitary cancer development with coexpression of the Fluc gene enabled longitudinal monitoring of tumor onset, progression, and response to therapy and may be used effectively for testing cancer prevention and treatment strategies on the basis of therapeutics that specifically target the retinoblastoma pathway. More recently, Momota and Holland55 have developed an imaging approach to measure the occurrence of nucleocytoplasmic trafficking of the androgen receptor and the split Rluc fragments. On delivery of DHT, there was restored bioluminescence signal intensity indirectly indicating the trafficking of the receptor to the nucleus. This was achieved by the imaging of genetically induced loss of RB control as a model of human gliomas.

### Imaging of Molecular Interactions or Events

Some interesting variations on standard reporter gene assays described previously have also been adapted recently for imaging of molecular interactions in the brains of mice. In particular, imaging interacting protein partners or protein trafficking in mice could pave the way for functional proteomics in whole animals and the assessment of dysfunctional signaling networks in diseased cells and could provide a tool for evaluation of new pharmaceuticals targeted to modulate protein-protein interactions and protein translocation.56 To this end, Kim et al57 have developed a genetically encoded bioluminescent indicator for imaging the nuclear trafficking of target proteins in vivo. The principle is based on reconstitution of split fragments of Renilla luciferase (Rluc) that are inactive in their split state. The N-terminal fragment of split Rluc is intentionally localized in the nucleus, whereas the C-terminal fragment joined to a particular protein (the androgen receptor in this example) is in the cytosol. Translocation of the receptor (on binding to 5a-dihydrotestosterone [DHT]) into the nucleus results in reconstitution of full-length Rluc and recovering its bioluminescence activity. Thus, imaging and quantifying the occurrence of nucleocytoplasmic trafficking of the androgen receptor was demonstrated after brain implantation of COS-7 cells cotransfected with the genes encoding the receptor and the split Rluc fragments. On delivery of DHT, there was restored bioluminescence signal intensity indirectly indicating the trafficking of the receptor to the nucleus.
reduced or inhibited on intraperitoneal injection of 2 agents, procymidone and polychlorinated biphenyls, supporting their likely antiandrogenic and neurotoxic effects, respectively. This study could provide a basis for a wide variety of imaging applications for screening drugs or neurotoxic compounds and testing them in preclinical animal models.

**Clinical Applications in Molecular Neuroimaging Using Reporter Genes**

One expectation of the ongoing developmental research in reporter gene expression imaging exemplified previously might be its straightforward translation from animal work to clinical practice. However, human applications present more theoretic and practical challenges than those in laboratory rodents. This is mostly because of the need for molecular probes to be biocompatible in humans, the presence of many physiologic/morphologic barriers to the delivery of genes and probes, and the need to develop special in vivo amplification strategies for low-level biologic events. Moreover, clinical imaging systems must be capable of obtaining high spatial/temporal resolution images and must be sensitive enough to detect these biologic processes. Because it is necessary to transduce living cells with imaging reporter genes, it follows that many of these practical requirements for successful implementation of reporter gene imaging in patients would mirror many of the logistic requirements and concerns in the field of human gene therapy. In addition, gene therapy is one of the main target areas of reporter gene imaging research because these imaging technologies are anticipated to be of significant help in monitoring transgene expression in a noninvasive manner.

Gene therapy has been one of the great yet unfulfilled promises of recent years. Yet, it has shown slow but steady progress thus far, with many of the obstacles becoming surmountable. Progress in this state of affairs will define mostly promises of recent years. Yet, it has shown slow but steady progress thus far, with many of the obstacles becoming surmountable. Progress in this state of affairs will define mostly

| Type of Genetically Engineered Model | Promoter Expression | Location of Model | Animal Method | Imaging | Application | Reference |
|------------------------------------|---------------------|------------------|---------------|--------|-------------|-----------|
| Transgenic | Estrogen receptor | Ubiquitous + brain | Mice | Fluc BLI | Study of estrogen control of growth, differentiation, and function of many systems; study of implications for estrogen-replacement therapy | 44 |
| Transgenic | GFAP | Brain | Mice | Fluc BLI | Dynamic monitoring of neuronal cell death | 45 |
| Transgenic | Smad binding element responsive to TGF-β signaling | Brain | Mice | Fluc BLI | Study of Smad2/3 activation in traumatic brain injury | 46 |
| Transgenic | Smad binding element responsive to TGF-β signaling | Brain | Mice | Fluc BLI | Study of Smad2 activation in neuronal degeneration | 47 |
| Transgenic | Serum amyloid A protein 1 | Ubiquitous + brain | Mice | Fluc BLI | Study of role of SAA1 induction in chronic inflammation associated with amyloid deposition | 48 |
| Gene targeting knockin | CMV | Ubiquitous + brain | Mice | GFP and RFP fluorescence | Study of alternative splicing regulation of FGFR-2 in the brain. | 49 |
| Transgenic | IκBα | Ubiquitous + brain | Mice | Fluc BLI | Study of regulation of IκBα expression and NF-κB transcriptional activity | 50 |
| Transgenic | Mouse GFAP | Brain | Mice | Fluc BLI | Study of meningitis and accompanying neuronal injury | 51 |
| Transgenic | c-fos, CMV | Ubiquitous + brain | Mice | Fluc BLI | Study of immediate-early genes involved in neural pathways linked to specific behaviors | 52 |
| Transgenic | Estrogen-responsive elements | Ubiquitous + brain | Mice | Fluc BLI | Study of activation of estrogen receptors and kinetics of gene activation by estrogenic compounds | 53 |
| Conditional recombinase knockout | Pro-opiomelanocortin | Pituitary | Mice | Fluc BLI | Study of spontaneous retinoblastoma pathway-dependent pituitary cancer and its response to doxorubicin | 54 |
| Transgenic | E2F1 | Brain | Mice | Fluc BLI | Imaging cell proliferation in gliomas with loss of RB control | 55 |

**Note:**—BLI indicates bioluminescence imaging; FGFR-2, fibroblast growth factor receptor-2; CMV, cytomegalovirus; GFP, green fluorescent protein; RFP, red fluorescent protein; IκBα, an inhibitor of nuclear transcription factor NF-κB, which regulates the expression of proinflammatory and cytotoxic genes; c-fos, an immediate early gene; RB, retinoblastoma protein.
expression of exogenous or endogenous genes to cells or tissue in humans by using imaging reporters for long-term imaging is a theoretic major hurdle at present and will remain so until the current practical challenges of human gene therapy discussed previously are addressed appropriately. Intensive ongoing efforts are also underway to develop alternative simpler strategies for potential future human applications, such as the delivery of circulating exogenous split reporter proteins into cells by using leader peptide sequences. On the other hand and unlike gene therapy, future clinical applications in cell therapy (eg, by using cell-mediated immunotherapy or stem cells) likely stand to benefit considerably and much sooner from reporter gene imaging, as is already clear from studies of cell trafficking in animal experiments. Indeed, Yaghoubi et al have recently demonstrated the first clinical experience in positron-emission tomography (PET) imaging of herpes simplex virus type-1–thymidine kinase (HSV1-tk)-expressing autologous cytolytic T lymphocytes directed at recurrent gliomas.

As with gene therapy, one of the challenges facing reporter gene expression imaging is to generate disease- or site-specific imaging strategies. Both the transductional targeting of the vector and the restriction of reporter gene expression solely to the target are potential avenues to follow once translated into clinical practice. Sufficient imaging probe would need to reach the target in vivo to achieve this specificity. Unlike MR imaging and bioluminescence imaging, both PET and SPECT use trace amounts (nonpharmacologic nanogram levels) of molecular probe to obtain images, as is the current practice for clinical practice far outweigh for now its exceedingly advantageous high sensitivity for detecting low-level biologic events. Similarly, the limitations of MR imaging preclude for now its applications in reporter gene imaging of the brain. These issues, therefore, tend to favor the use of PET and SPECT imaging as a viable compromise for clinical implementation of this molecular imaging strategy in neuroimaging, particularly when considering the many merits of these 2 techniques. However, biologic and biophysical factors involved in the biodistribution of reporter probes that are potentially applicable to clinical imaging will also need to be studied carefully as they are scaled up from small animal imaging.

Regrettably, the molecular probes for the HSV1-tk enzyme (both of those based on radiolabeled uracil nucleosides and acycloguanosine derivatives) barely penetrate the intact blood-brain barrier (BBB). The BBB is a selective barrier formed by endothelial cells lining cerebral microvessels. It acts as a de facto “physical barrier” on account of complex tight junctions between adjacent endothelial cells, forcing most molecular traffic to take a transcellular route across the BBB, rather than moving paracellularly through the junctions, as in most endothelia. This effectively filters most ionized water-soluble molecules >180 Da in molecular weight. Most molecular probes for HSV1-tk are based on the structure of ganciclovir. This has a molecular weight of 255 Da, and it only achieves a concentration in the brain of about 50% of the plasma level. 18F-labeled acycloguanosine derivatives are heavier and usually with extra methyl and fluoro side chains added to the ganciclovir structure.

Not surprisingly, Hespers et al have found in previous biodistribution studies that the uptake of 9-[(3-[18F] fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG) in brain tissue is approximately eightfold lower than the level of FHPG in plasma, reflecting this restricted passage through the intact BBB. On the other hand, the disrupted blood-tumor barrier has been shown in some studies to allow passage of similar probes in experimental rodent intracranial tumors, but this is not a consistent observation (see below findings in the clinical setting). As well, the permeability of the BBB may be altered during cerebral infection (eg, herpes simplex encephalitis) due to the release of chemical mediators such as bradykinin, arachidonic acid, histamine, and free radicals. Attempts to modulate the permeability of the BBB pharmacologically have been undertaken to enhance chemotherapeutic drug delivery within the brain. LeMay et al have previously demonstrated that the vasodilatory bradykinin analog RMP-7 increases brain tumor permeability to ganciclovir. It remains to be investigated whether the use of osmotic disruption or RMP-7 may possibly increase delivery of other HSV1-tk substrates across the BBB for molecular neuroimaging purposes.

Unfortunately, once injected systemically, the promiscuous tropism of certain viruses does limit cell-specific gene delivery by these vectors. Viral engineering strategies could ultimately benefit reporter gene expression in the clinical setting, especially to address the tropism of adeno viral vectors. Therefore, local delivery of imaging genes to the brain has been the only means of tissue transduction in the 2 preliminary clinical neuroimaging studies reported so far. Jacobs et al intraoperatively infused liposome vectors carrying the reporter HSV1-tk gene directly into tumors during a clinical phase I/II gene therapy trial of 5 patients with...
reccurent glioblastoma. Noninvasive primary end point (indirect) molecular imaging of the transduced “tissue dose” of vector-mediated therapeutic gene expression was performed by using the molecular probe 2’-fluoro-2’-deoxy-l-beta-D-arabinofuranosyl-5-ido-uracil (124I-FIAU) and PET. The imaged “tissue dose” of therapeutic gene expression was also correlated with the induced therapeutic effect by secondary end point molecular imaging of the metabolic activity and proliferative activity of the tumors by using $[^{18}F]$fluorodeoxyglucose ($^{18}$F-FDG) and $[^{11}C]$methionine ($^{11}$C-MET), respectively, and PET. One of the 5 patients demonstrated $^{124}$I-FIAU accumulation that was significantly above the prevector baseline and therefore consistent with successful imaging of HSV1-tk gene expression in gene therapy in man. Moreover, their findings possibly indicate that PET would be a useful tool to monitor transgene expression in gene therapy clinical trials by using viral vectors.

These early clinical examples also demonstrate the kind of synergy necessary between direct molecular imaging (eg, by using $^{18}$F-FDG and $^{11}$C-MET) and reporter gene methods in successful establishment of safe and effective gene therapy protocols in clinical practice. In another recent study, Dempsey et al attempted, for the first time, to image expression of HSV1716 during oncolytic viral therapy in human malignant glioma. $^{131}$I-FIAU brain SPECT imaging was undertaken in 8 patients receiving intratumoral injection of virus, but no molecular probe accumulation was detected in these treated gliomas. The authors discussed the many factors that may have contributed to this lack of imaging signal intensity, including impermeability of the BBB, inconsistent disruption of BBB, short half-life of $^{131}$I, lower sensitivity of SPECT compared with PET, the use of weak promoters, the need for more sensitive molecular probes, possible insufficient viral replication, and potential for improved administration of virus (eg, by using convection enhanced delivery). Nonetheless, this study was useful in highlighting the possible limitations of this technique and the many potential areas that need to be investigated in future research.

Future Outlook

In this article, we discussed the principles and recent technologic advances in molecular imaging of reporter gene expression in the brain. This approach is emerging as a valuable tool for monitoring the expression of genes in laboratory animals and humans. Further development of newer (eg, Gaussia luciferase) and more sensitive and selective reporters (eg, red-shifted Rluc, with greater stability and higher light emission than native Rluc), combined with improvements in detection technology, will consolidate the position of reporter gene imaging as a versatile method for understanding of intracellular biologic processes and the molecular basis of neurologic disorders.

Many developments in reporter gene expression imaging are anticipated during the next decade. Significant conceptual and technologic advances will most likely be seen across the 5 main general requirements for molecular imaging discussed previously and in greater detail elsewhere,—that is, knowledge of molecular targets, availability of molecular probes, overcoming delivery barriers, developing amplification strategies, and availability of appropriate instrumentation. In particular, new strategies to circumvent the normal BBB or that target a blood-tumor barrier by the use of novel carrier vehicles (eg, in rabbits, various nutrient transporters continue to be tested across the blood-retinal barrier [used as a model of the BBB] to enhance drug bioavailability across membranes with poor permeability) and local vasodilation or osmotic opening all merit attention, as well as the design of newer reporter gene/probe systems tailored to molecular neuroimaging. As an example of the latter, Majumdar et al have tested modified dipeptide monoester ganciclovir prodrugs for their greater solubility and permeability. On the other hand, Ma-
grassi et al.2 exploited the fact that HSV1-tk is not enantioselective and can therefore efficiently phosphorylate both D and L enantiomers of β-thymidine. Using autoradiography, they showed that tritiated L-β-thymidine is selectively retained to a significant extent in experimental intracranial gliomas. It has the advantage of generating less-toxic metabolites than with use of conventional HSV1-tk probes; and with appropriate radioisotopic labeling (eg, with 11C) of l-thymidine, it might be possible to adapt it for future use in PET studies of the brain.

Another study that exemplifies a search for new reporter systems with capabilities of imaging processes behind an intact BBB is that of Doubrovin et al,83 who investigated *Escherichia coli* xanthine phosphorybosyltransferase for nuclear imaging with radiolabeled xanthine. Again, by using autoradiography, they found that 14C xanthine was capable of specific accumulation in transplanted infiltrative brain tumors. These and future similar innovations bode well for more widespread experimental and potential clinical applications of reporter gene imaging in the brain.

The merger of molecular biology and medical imaging is facilitating rapid growth of this new field by providing methods to monitor an ever-increasing number of cellular/molecular events adapted from conventional molecular assays, including reporter gene assays. Further developments will provide us with the ability to perform simultaneous imaging of multiple molecular events in 1 population of cells in living subjects. This may be attainable by combining 2 or more of the previously described strategies for gene marking and imaging the trafficking of cells with those entailing linked expression of an imaging gene to an endogenous promoter or to an exogenous therapeutic gene. As such, in these applications, it is foreseeable that 1 reporter may reveal the spatial distribution of cells and whether they have reached a specific target, and another reporter may indicate whether a certain gene becomes upregulated at this site or if a more complex interaction occurs. These endeavors will be aided by the availability of multiple fusion reporter constructs (eg, those that combine PET/bioluminescence/fluo- rescence imaging capabilities in 1 gene),84 the use of which should accelerate the validation of reporter gene approaches developed in cell culture for translation into preclinical models and subsequent clinical imaging of neurologic disorders. With continued rapid advancements in this field, the experimental and clinical neurosciences stand to gain considerably from noninvasive molecular imaging of the expression of multiple fused reporter genes by using multiple imaging techniques.85 These approaches are likely to play an increasingly important role in defining molecular events in the field of cancer biology, cell biology, and gene therapy within the central nervous system.

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