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Review

핵의학적 기법을 이용한 유전자 치료 영상법

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Monitoring Gene Therapy by Radionuclide Approaches

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Molecular imaging has its root in nuclear medicine and gene therapy monitoring. Therefore, recent progress in the development of non-invasive imaging technologies, particularly nuclear medicine, should allow molecular imaging to play a major role in the field of gene therapy. These tools have recently been validated in gene therapy models for continuous quantitative monitoring of the location, magnitude, and time-variation of gene delivery and/or expression. This article reviews the use of radionuclide imaging technologies as they have been used in imaging gene delivery and gene expression for gene therapy applications. The studies published to date lend support that noninvasive imaging tools will help to accelerate pre-clinical model validation as well as allow for clinical monitoring of human gene therapy. (Nucl Med Mol Imaging 2006;40(2):96-105)

Key Words : molecular imaging, gene therapy, radionuclide imaging, positron emission tomography (PET)

Introduction

Diagnostic imaging technologies have been used to try to monitor transgene expression for gene therapy using magnetic resonance imaging (MRI), optical imaging, and radionuclide imaging techniques including positron emission tomography (PET) and single photon emission computed tomography (SPECT).[1-5] Reporter genes with optical signatures (e.g., fluorescence and bioluminescence) are low-cost alternatives for real-time analysis of gene expression in small animal models. Fluorescence imaging uses a fluorescent protein such as green fluorescent protein (GFP) that is excited with external illumination and the emission is subsequently detected.[6] Excitation and emission wavelengths in the range of 500 nm (e.g., GFP) have limited penetration in mammalian tissues (1-5 mm) and autofluorescence of tissues can result in poor signal-to-noise.[4] Although red shifted mutants of GFP (RFP) has been known to have an advantage over GFP that red light penetrates tissues more efficiently than green,[7] it still have serious limitation to utilize in larger animals. Bioluminescent photoproteins such as luciferase have been used as reporter proteins in living animals.[2,8,9] Firefly luciferase (FL) catalyzes D-Luciferin to produce oxyluciferin in the presence of oxygen, cofactors, Mg^{+2}, and ATP to produce light with peak at 562 nm. Renilla luciferase (RL) catalyzes the oxidation of coelenterazine in presence of oxygen to generate a flash of blue luminescence with a peak wavelength at 482 nm. The advantage of bioluminescence is the minimal background noise, since luciferase is not a natural constituent of mammalian organisms. Bioluminesence based approaches currently lack detailed tomographic information and are limited to relatively small animals.[10-12] A newer approach to fluorescence imaging of deeper structures uses fluorescence-mediated tomography.[13] The subject is exposed in an imaging chamber to continuous wave or pulsed light from different sources, and detectors arranged in a spatially defined order capture the emitted light. Mathematical processing of this information results in a reconstructed tomographic image. Fluorescence-mediated tomography is

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still in its infancy, requiring extensive mathematical validation prior to routine implementation. The advantage of MR for imaging of gene expression are the excellent three-dimensional spatial resolution (tens of μ m range) at imaging. Because of the indirect nature of enhancement produced by MR contrast agents, much higher concentration of injected material, on the order of 10-100 micromolar concentrations and higher, generally are necessary to produce sufficient image contrast.[2,4,5,10,14] The low sensitivity often entails long imaging times and consequently slow data acquisition.[5] While Magnetic Resonance Spectroscopy (MRS) does not usually produce three-dimensional images, the technique does provide accurate measurement of gene expression in short time frames and may eventually be harnessed to produce true spatial images but at much poorer spatial resolution than MRI. Radionuclide imaging with PET and SPECT has been used to characterize enzyme activity, receptor/transporter status and biodistribution of various radiolabeled substrates (tracers).[15] For these reason, it has made the most significant progress for imaging gene therapy by monitoring gene delivery and identifying therapeutic and/or reporter gene expression in living subjects. While sensitivity in PET imaging is high (as little as 10^{-11}-10^{-12} M of tracer can be detected) and the speed of imaging is relatively rapid (minutes), these techniques lack micrometer spatial resolution (1-2 mm with micro PET).[4,5,16] An alternative approach to PET is SPECT imging. While the sensitivity of single-photon system is intrinsically about one-two orders of magnitude less than PET systems, required radiopharmaceuticals and imaging systems are more readily available. Further details of the instrumentation available and relative advantages between the various types of imaging instrumentation may be found elsewhere.[2,3,17]

In the following sections, we will review radionuclide imaging technologies for monitoring gene delivery or transgene expression. Much of the current focus of molecular imaging in gene therapy is directed towards oncological applications, however preliminary studies for cardiovascular and neurolgical applications have also been reported.

Oncology

1. Imaging the vector utilized for gene delivery

To assess the efficiency of vector delivery, radionuclide imaging can be used to look at the distribution of the radiolabeled vector itself. The ideal gene therapy paradigm for brain tumors may consists of a combination of intratumoral injection and intra-arterial administration of vectors bearing therapeutic transgenes. In previous studies, herpes simplex virus (HSV) was radiolabeled with lipophilic [^{111}In]-oxine complex to be administered to intracerebral glioma bearing rats. Intracarotid injection of radiolabeled HSV revealed low efficiency of viral uptake in the tumor (0.10% of the injected dose per gram of tissue) at 1 hour. When animals received virus injections stereotactically into the tumor, 71.3±35.0% of the total dose was found in the tumor at 24h.[18] An alternative labeling approach based on [^{99m}Tc] labeled recombinant adenovirus serotype 5 knob (Ad5K) has also been validated.[4] Imaging data for the hepatic uptake studies were in agreement with biodistribution determined by removing and measuring tissues. Recently, the potential of labeling adenovirus with [^{99m}Tc] or [^{124}I] has been investigated to study viral biodistribution and demonstrated stable labeling with [^{99m}Tc] using an Isolink carbonyl kit[19] and [^{124}I] using the standard iodogen method[20] without loss of viability or infectivity. This allows imaging viral biodistribution and reporter gene expression.

Imaging of non-viral vector delivery has been studied for direct visualization of the distribution of double stranded DNA (pCMV-GFP). The generic structure of the probe comprises three elements: (1) a peptide-based chelate that binds the [^{99m}Tc]; (2) a positively charged linker for binding to DNA phosphodiester backbone; (3) an intercalating psoralen group.[21] The formation of a stable complex between the probe and the DNA is achieved by ultraviolet crosslinking of psoralen and DNA. The feasibility of imaging the delivery of plasmid DNA was shown in normal and tumor-bearing animals using gamma camera imaging. Non-viral genetic vector, pegylated liposome, was also radiolabeled to monitor the gene delivery. A preliminary study demonstrated gamma camera images after intravenous infusion of [^{111}In]DTPA-labeled

pegylated liposome revealed high level of accumulation in the head and neck cancer lesions of patients,[22] and therefore strongly supported the use of pegylated liposome as a targeting vehicle of therapeutic gene for solid tumors. The imaging of gene delivery *in vivo* could serve as a general predictor of the ability of the viral or non-viral vectors to reach the tissue(s) of interest. However, mere visualization of exogenous DNA accumulation at a certain site in the body might not correlate with the expression levels of desired gene product.

2. Imaging cell trafficking

1) Direct Cell Labeling

In vivo imaging of cell trafficking has been investigated in many immunological and oncological studies to track the selective recruitment and time of arrival and departure of specific cells. Direct cell labeling with a radionuclide has been used for many years to track cells over periods of several hours, or even days, such as [^{111}In] ($T_{1/2}$=2.8 days) and [^{99m}Tc] ($T_{1/2}$=6 hours) for SPECT or [^{18}F]FDG ($T_{1/2}$=2 hours) and [^{64}Cu] ($T_{1/2}$=12.7 days) for PET.[23-29] For example, Botti et al.[27] used [^{99m}Tc]HMPAO, [^{111}In]oxine and [^{18}F]FDG to follow the migratory pattern of activated lymphocytes. Paik et al.[28] improved the [^{18}F]FDG labeling of monocytes by incubating the cells in the presence of insulin. Rat glioma (C6) cells and lymphocytes were radiolabeled using [^{64}Cu]pyruvaldehyde-bis(N^4-methylthiosemicarbazone) ([^{64}Cu]PTSM) and imaged with microPET in living nude mice. MicroPET images indicated trafficking of tail-vein-injected C6 cells to the lungs and liver, and transient splenic accumulation of lymphocytes at 3.33 h postinfusion. While direct cell labeling has many attractive features such as simplicity, the drawbacks include radiotoxicity effects, loss of label from cells, dilution of signal from cell division, and lack of information on cell function or viability. In particular, the incomplete information regarding cell survival with direct labeling has been the impetus behind developing reporter genes to track cells.[30]

2) Reporter Imaging Technology

The approach of utilizing a reporter gene, whose expression can be quantified by the imaging signal, is attractive for the evaluation of survival simply because only viable cells will express the reporter. Reporter gene imaging is also being used to follow the specific localization and expansion of adoptively transferred immune T lymphocytes to the antigen-positive tumor and other sites within the animal.[31,32] This approach can be used to assess the effects of immunomodulatory agents intended to potentiate the immune response to cancer, and can also be useful for the study of other cell-mediated immune responses, including autoimmunity.

In a slightly different approach to assess tumor cell trafficking, neoplastic cells can be transfected with a reporter gene and then implanted into recipient subjects. Ponomarev et al.[33] monitored TCR-mediated T-cell activation. Jurkat cells were transduced with a retroviral vector encoding a HSV1-TK-GFP fusion protein whose expression was driven by a NFAT-sensitive promoter. When stimulated in vivo by either tetradecanoyl phorbol acetate and ionomycin or anti-CD3, the cells expressed the HSV1-TK-GFP reporter. Subcutaneous xenografts of these tumors in nu/nu mice (which have no mature thymocytes) were relatively devoid of signal following radiolabeled FIAU injection. Following intravenous administration of anti-CD3 and anti-CD28 antibodies, however, a signal was easily revealed by the PET scanner. This is the first example of the use of PET reporter genes in the noninvasive measurement of T-cell activation in a live animal.

3. Imaging Therapeutic Gene Expression

Radionuclide imaging technologies, especially PET and SPECT can play a significant role in imaging gene expression using diverse reporter genes and reporter probes. A reporter gene can be introduced into target tissue(s) by various methods including viral and non-viral delivery vectors. If the promoter leads to transcription of the reporter gene, then translation of the imaging reporter gene mRNA leads to a protein product which can interact with the imaging reporter probe (administered in trace amounts for PET/SPECT and sometimes referred to as a tracer). This interaction may be based on intracellular enzymatic conversion of the reporter probe with retention of the

metabolite(s), or a receptor-ligand based interaction. Examples of intracellular reporters include herpes simplex virus type1 thymidine kinase (HSV1-tk) and its mutant gene (HSV1-sr39tk).[34] Note that HSV1-tk or HSV1-sr39tk refers to the genes and HSV1-TK or HSV1-sr39TK refers to the respective enzymes. Substrates that have been studied to date as PET reporter probes for HSV1-TK can be classified into two main categories - pyrimidine nucleoside derivatives [e.g., 5-iodo-2'-fluoro-2'-deoxy-1-β-D-arabinofuranosyluracil (FIAU)] and acycloguanosine derivatives [e.g., 9-(4-fluoro-3-hydroxymethylbutyl)guanine (FHBG)], and have been studied in terms of sensitivity and specificity.[35,36] Examples of reporters on or in the cell surface in the form of receptors include the dopamine 2 receptor (D_2R),[37] receptors for human type 2 somatostatin receptor (hSSTr2)[38] and the sodium iodide symporter (NIS).[39,40] Among these reporter genes, the HSV1-tk gene may alter the cellular behavior towards apoptosis by changes in the dNTP pool,[41] and receptors may result in second messenger activation such as triggering of signal transduction pathways. For the D_2R system a mutant gene has been studied which shows uncoupling of signal transduction but preservation of affinity of receptor for tracer ligand.[42]

The reporter gene can itself be the therapeutic gene or can be coupled to the therapeutic gene.[16] In the former approach, the reporter gene and therapeutic gene are one in the same. For example, anti-cancer gene therapy using HSV1-tk and ganciclovir (GCV) can be coupled with imaging of the accumulation of radiolabeled probes ([^{18}F]FHBG or [$^{131/124}$I]FIAU).[35,36,43] Jacobs et al.[44] used [^{124}I]FIAU PET imaging of humans in a prospective gene-therapy trial of intratumorally infused liposome-gene complex (LIPO-HSV1-tk) followed by GCV administration in 5 recurrent glioblastoma patients. These preliminary findings showed that [^{124}I]FIAU PET is feasible and that vector-mediated gene expression may predict a therapeutic effect. Recently, sodium/iodide symporter (NIS) which facilitate the uptake of iodide by thyroid follicular cells is also being applied in radioiodide gene therapy.[39,45] The conventional radioiodide or [^{99m}Tc]pertechnetate scintigraphy has been used to directly monitor NIS expression.[45-48] NIS has many advantages as an imaging reporter gene that include wide availability of its substrates, well-understood metabolism and clearance of these substrates in the body, and no likely interaction with the underlying cellular biochemistry. Since the Iodine is not trapped, issue of efflux have to be optimized but initial studies show significant promise. Further studies are needed with regard to NIS as an imaging reporter gene.

A second approach involves *indirect* imaging of therapeutic transgene expression using expression of a reporter gene which is coupled to a therapeutic transgene of choice. This strategy requires proportional and constant co-expression of both the reporter gene and the therapeutic gene over a wide range of transgene expression levels. An advantage of this approach is that it provides for a much wider application of therapeutic transgene imaging, because various imaging reporter genes can be coupled to various therapeutic transgenes while utilizing the same imaging probe each time. Linking the expression of a therapeutic gene to a reporter gene has been validated using PET through a variety of different molecular constructs. Examples include fusion approaches,[49-51] bicistronic approaches using internal ribosomal entry site (IRES),[52-54] dual-promoter approaches,[55,56] a bidirectional transcriptional approach,[57] and a two vector administration approach.[58] An advantage of the fusion gene approach is that the expression of the linked genes is absolutely coupled (unless the spacer between the two proteins is cleaved). However, the fusion protein does not always yield functional activity for both of the individual proteins and/or may not localize in an appropriate sub-cellular compartment. Although the IRES sequence leads to proper translation of the downstream cistron from a bicistronic vector, translation from the IRES can be cell type specific and the magnitude of expression of the gene placed distal to the IRES is often attenuated.[53] This can lead to a lower imaging sensitivity, and methods to improve this approach are currently under investigation.[54] Two different genes expressed from distinct promoters within a single vector (dual-promoter approach) may avoid some of the attenuation and tissue variation problems of an IRES-based approach.[55] The potential problem of this approach is the expression of the two genes may become uncoupled if the two identical promoters have different transcriptional activity based on

where the vector integrates into the host genome or if a mutation occurs in one or both promoters that changes transcriptional activity. A bi-directional transcriptional approach utilizes a vector in which the therapeutic and the reporter genes are driven by each minimal CMV promoter induced by tetracycline-responsive element (TRE), transcribing separated mRNA from each gene which would then be translated into separate protein products.[57] This system also avoids the attenuation and tissue variation problems of the IRES based approach and may prove to be one of the most robust approaches developed to date. It is limited by the fact that a fusion protein also needs to be co-expressed, but future vectors should be able to encode for both the fusion protein and the bi-directional transcriptional system on a single vector. Another way to image both the therapeutic and reporter genes can be through administration of two separate vectors, by cloning of the therapeutic and reporter genes in two different vectors but driven by same promoter. This system may eliminate the need for making a new construct for each therapeutic gene, and has been validated through the expression of two PET reporter genes and showed good correlation.[58] However, it is important to realize that *trans* effects between two promoters can potentially affect reporter gene expression and that not all cells may be equally infected with the PET reporter gene vector and therapeutic gene vector.

Tumor-restricted gene expression through tissue specific transcriptional targeting is an attractive approach for gene therapy. It has been demonstrated that gene expression of highly efficient gene therapy vectors can be targeted to tumors using cell-type or tissue-type specific promoter elements. Approaches have also been developed to image the transcriptional regulation of PET reporter gene in living animals.[33,57,59] For example, transcriptional regulation at the level of induction has been reported in living mice using two PET imaging reporter genes (HSV1-tk and D_2R) under the control of a tetracycline-inducible promoter.[57] Using PET, correlative expression of both reporters after doxycycline treatment was measured in animals harboring stably transfected tumor. Low levels of imaging reporter gene expression owing to relatively weak tissue-specific promoters were circumvented with VP16 transactivating domains fused to yeast GAL4 DNA-binding domains. This two step transcriptional amplification (TSTA) system was valuable in demonstrating PSE or CEA driven reporter gene expression *in vivo* using HSV1-sr39tk or HSV1-tk under the control of GAL4-responsive elements.[60-65] Further studies are necessary to link this system to amplify both therapeutic and reporter gene expression. These approaches hold significant promise for development of tissue specific vectors with high levels of gene expression.

The lytic properties of herpes, adeno or Newcastle viruses are also being tailored for destruction of various tumors. Although these oncolytic viruses are promising agents for treatment of malignancy due to their direct, selective toxicity for tumor cells, it is not easy to document viral replication in living subjects, as serial tissue sampling was required to assess viral titers over time. [^{124}I]FIAU PET scanning was capable of distinguishing a half-log difference between viral doses and was able to document viral proliferation in xenograft tumor infected by oncolytic HSV infection.[66,67] This PET data might provide a new direction for evaluating viral infection and proliferation in future clinical trials involving oncolytic viral therapy.

Currently, Penuelas et al.[68,69] monitored thymidine kinase gene expression after intratumoral injection of a first-generation recombinant adenovirus in patients with hepatocellular carcinoma using ^{18}F-FHBG PET. (Fig. 1) In this study, transgene expression in the tumor was dependent on the injected dose of the adenovirus and was detectable in all patients who received $\geq 10^{12}$ viral particles. This study is the pioneering trials on clinical application of human gene therapy imaging along with colon cancer[69] and glioblastoma[44] patients trials.

Cardiovascular Disease

Gene therapy holds much promise as a potential treatment for various cardiovascular disease. These treatment include prevention of restenosis after angioplasty, promotion of angiogenesis, and treatment of end-stage heart failure.[70]

Initial studies using autoradiography detected uptake of [^{125}I]FIAU in rat myocardium transduced with adenoviral-mediated HSV1-tk reporter gene. The authors hypothesized

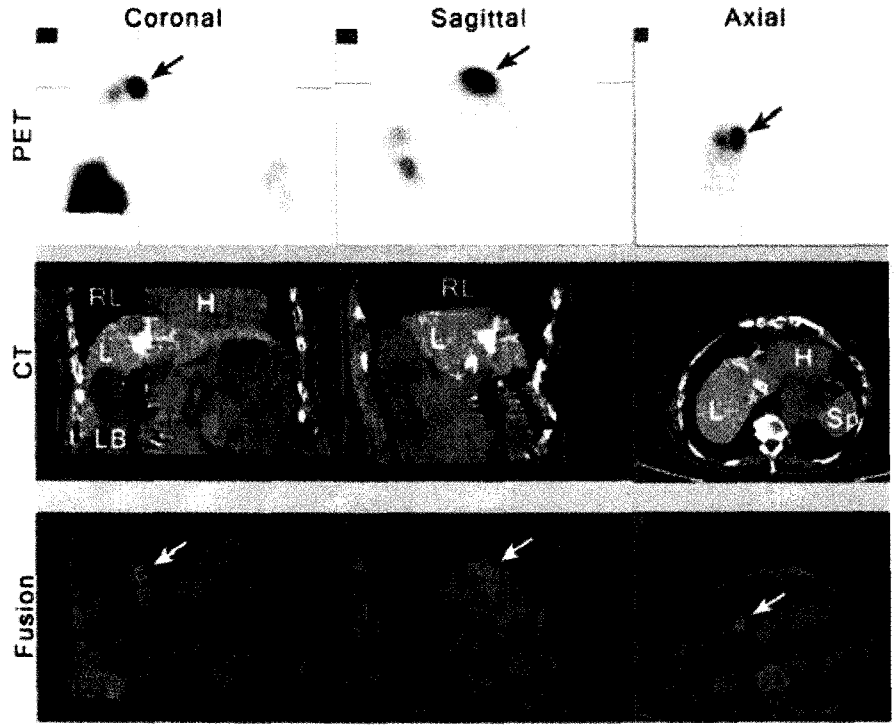

Fig 1. PET imaging of adenoviral-mediated transgene expression in liver cancer patients. PET-CT imaging of HSV1-tk transgene expression in humans. Columns 1 to 3 show the 5-mm-thick coronal, sagittal, and transaxial slices, respectively, from a (18F)FHBG-PET-CT study in patient 5. All sections are centered on the treated tumor lesion (dotted lines in the CT images) and show (18F)FHBG accumulation at the tumor site (arrows). Anatomicmetabolic correlation can be obtained by fused PET-CT imaging. The white spots on the liver seen on the CT images correspond to lipiodol (arrowheads) retention after transarterial embolization of the tumor and a transjugular intrahepatic portosystemic shunt (★). Tracer signal can be seen in the treated lesion (arrows), whereas no specific accumulation of the tracer can be seen in the necrotic, lipiodol-retaining regions around it. H, heart; L, liver; LB, large bowel; RL, right lung; Sp, spleen. Image used with permission.[68,69]

that *in vivo* cardiac gene imaging is feasible and may eventually be used for the noninvasive monitoring of gene therapy.[71] The first demonstration of cardiac reporter gene imaging in living subjects was reported with FL bioluminescence imaging. This study demonstrated the feasibility of imaging the location, magnitude, and time course of cardiac reporter gene expression in living rats.[72] The optical study was validated in a clinically relevant microPET. Rat myocardium was transduced with adenovirus carrying HSV1-sr39tk. The presence of [^{18}F]FHBG uptake in microPET images was confirmed by gamma counting and the presence of HSV1-sr39TK protein by thymidine kinase enzyme assay while utilizing myocardial tissue samples.[73] More detailed quantitative microPET studies have also been performed using this same model.[74] Another study has been performed to construct bicistronic vectors containing 2 PET reporter genes (CMV-D2R80a-IRES-HSV1-sr39tk).[75] Longitudinal [^{18}F]-FESP and [^{18}F]-FHBG imaging of experimental rats revealed a good correlation between the cardiac expressions of the 2 PET reporter genes reflecting that the IRES-based bicistronic adenoviral vector can potentially be used in conjunction with PET for indirect imaging of therapeutic gene expression by replacing 1 of the 2 PET reporter genes with a therapeutic gene of choice. Further studies have been performed to construct bicistronic vectors containing both therapeutic (e.g., VEGF) and PET reporter genes.[76] This was the first cardiac study to validate the novel approach of linking a therapeutic gene (VEGF121) with a PET reporter gene (HSV1-sr39tk). Findings were as follows: (1) the dual-cassette adenoviral vector could efficiently transfect rat embryonic cardiomyo-

blasts (in vitro) and rat myocardium (in vivo), with strong correlations between the 2 genes; (2) noninvasive imaging showed that adenovirus-mediated gene expression peaks at 1 to 3 days, lasts ≈ 2 weeks, and is not aided by repeated injections; (3) in vivo images of HSV1-sr39tk reporter gene activity correspond well with traditional invasive techniques such as ex vivo gamma counting, enzyme assays, and immunohistochemistry; (4) VEGF121 gene transfer induces formation of new capillaries and small blood vessels as determined by CD31 and α -SMA stains, respectively; and (5) under conditions of the present study, the microscopic level of neovasculature does not translate into significant changes in clinically relevant physiological parameters such as myocardial contractility, perfusion, and metabolism.

The concept of stem cell trafficking imaging using a reporter gene approach has been applied in cardiac molecular imaging research in which embryonic cardiomyoblasts expressing HSV1-sr39tk and/or FL were monitored non-invasively after implantation into myocardium.[77] The location, magnitude, and survival duration of tranplanted cells were monitored noninvasively using PET and bioluminescence optical imaging. In vivo imaging findings demonstrated that large fractions of donor cells were dead first 1 to 4 days after implantation. Therefore, the efficacy of immunosuppressive modulators on cell survival has been systemically explored by optical bioluminescence technique. Dexamethasone, mycophenolate and tacrolimus can improve cell survival during the first week, but not by the second week.[78,79]

Miscellaneous Diseases

Both the HSV1-tk/HSV1-sr39tk and the D_2R PET reporter genes are not optimal for most central nervous system application. All of the reporter probes for HSV1-tk/HSV1-sr39tk developed to date show very poor penetration across blood-brain barrier (BBB). Therefore, imaging with any of these reporter probes may be useful only if some BBB disruption is present (e.g., brain tumors). The D_2R reporter gene with [^{18}F]FESP can work with brain-specific applications but only if reporter gene expression is not in the vicinity of normal D_2R expression in the striatum.

Recently, in Parkinson's disease research, aromatic-amino-dopadecarboxylase (AADC) gene was delivered to striatum as a therapeutic gene via an adeno-associate viral vector (AAV) and its expression was imaged by [^{18}F] fluoro-m-tyrosine ([^{18}F]FMT) PET. The AADC tracer, [^{18}F]FMT, is both decarboxylated and stored in striatal neurons of monkeys providing a method for *in vivo* visualization of gene expression in the brain.

Cellular radiolabeling techniques can be useful to assess the biodistribution of various cells for cellular therapy applications. Hepatocyte transplantation has been shown to provide temporary liver function in acute hepatic failure and various metabolic diseases. [^{111}In] labeled hepatocytes was useful for the short-term noninvasive analysis of the biodistribution of transplanted hepatocytes. In another study, myoblasts were radiolabeled with [^{99m}Tc]bis (N-ethoxy, N-ethyl)dithiocarbamate (NOEt) and injected to assess the biodistribution of these cells in Duchenne type human muscular dystrophy model.[80]

Future Prospects

Molecular imaging strategies associated with gene therapy will likely expand significantly over the next few years as gene therapy continues to evolve. The explosion in genetic engineering is expected to generate more robust gene transfer vectors, both viral and non-viral. Bi-cistronic/ Bi-directional vectors which can be easily modified, and tissue specific amplification techniques will likely expand. Continued refinements in chemistry of molecular probe development should give rise to a new generation of probes with greater sensitivity and specificity. Advances in detector technology and image reconstruction techniques for PET should help to produce a newer generation of imaging instruments with better spatial resolution, sensitivity, and significantly improved throughput time. Multimodality reporter gene approaches so that gene therapy investigators may readily move between the various technologies should help to also test various pre-clinical models. It will be very important for pre-clinical imaging strategies to be extended into patient studies where gene therapy is directly or indirectly monitored

throught the use of state-of-art imaging. Currently, human trial with T cells and dendritic cells expressing HSV1-tk reporter gene and consecutive [^{18}F]FHBG PET imaging studies have been approved by FDA (communicated with Dr. Gambhir). Ultimately, all of the imaging technologies of gene delivery and/or expression will be used as an early measure of successful gene therapy in patients.

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