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Review

PET 리포터 유전자를 이용한 이행성 연구

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Translational Imaging with PET Reporter Gene Approaches

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Recent progress in the development of non-invasive imaging technologies continues to strengthen the role of biomedical research. These tools have been validated recently in variety of research models, and have been shown to provide continuous quantitative monitoring of the location(s), magnitude, and time-variation of gene delivery and/or expression. This article reviews the use of PET technologies as they have been used in imaging biological processes for molecular imaging applications. The studies published to date demonstrate that noninvasive imaging tools will help to accelerate pre-clinical model validation as well as allow for clinical monitoring of human diseases. (Nucl Med Mol Imaging 2006;40(6):279-292)

Key Words: molecular imaging, positron emission tomography, gene expression, cell trafficking, gene therapy

Introduction

Present imaging technologies rely mostly on non-specific morphological, physiological, or metabolic changes that differentiate pathological from normal tissue rather than identifying specific molecular events (e.g. gene expression) responsible for disease. Molecular imaging usually exploits specific molecular probes as the source of image contrast. This change in emphasis from a non-specific to a specific approach represents a significant paradigm shift, the impact of which is that imaging can now provide the potential for understanding of integrative biology, earlier detection and characterization of disease, and evaluation of treatment. Massoud et al.[1] suggested several important goals in molecular imaging research, namely: (1) To develop non-invasive in vivo imaging methods that reflect specific molecular processes such as gene expression, or more complex molecular interactions such as protein-protein interactions; (2) To monitor multiple molecular events near-simultaneously; (3) To follow trafficking and targeting of cells; (4) To optimize drug and gene therapy; (5) To image drug effects at a molecular and cellular level; (6) To assess disease progression at a molecular pathological level; and (7) To create the possibility of achieving all of the above goals of imaging in a rapid, reproducible, and quantitative manner, so as to be able to monitor time-dependent experimental, developmental, environmental, and therapeutic influences on gene products in the same animal or patient.

Molecular imaging has its roots in nuclear medicine. Nuclear medicine is focused on characterizing enzyme activity, receptor/transporter status and biodistribution of various radiolabeled substrates (tracers). Positron emission tomography (PET) imaging is attractive because it provides high resolution and is more sensitive than gamma camera imaging (by at least a logarithm). Thus, it provides quantitative information about the distribution of the tracer that is immediately translated into concentration of the tracer into the tissue of interest. In addition to dramatic advances in new high-resolution positron emission tomography (PET) scanners for small animal imaging, related progress in molecular and cell biology techniques, and in the development of specific imaging probes have

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contributed the rapid expansion of nuclear medicine techniques to the study of disease biology. These advances are now being explored to characterize molecular events and disease processes such as molecular interaction, gene expression, cell trafficking, and apoptosis. Here, we focus on the use of PET molecular imaging to study reporter gene expression.

Imaging Reporter Gene

Molecular biologists have used reporter genes both in vitro and in vivo for over a decade to monitor gene expression. Imaging of gene expression in living subjects can be directed either at genes externally transferred into cells of organ systems (transgenes) or at endogenous genes. Most current applications of reporter gene imaging are of the former variety. By adopting state-of-the-art molecular biology techniques, it is now possible to better image cellular/molecular events. One can also engineer cells that will accumulate imaging probes of choice, either to act as generic gene 'markers' for localizing and tracking these cells, or to target a specific biological process or pathway. In the last few years there has been a veritable explosion in the field of reporter gene imaging, with the aim of determining location, duration, and extent of gene expression within living subjects[1-3].

Reporter genes are used to study promoter/enhancer elements involved in gene expression, induction of gene expression using inducible promoters, and endogenous gene expression through the use of transgenes containing endogenous promoters fused to the reporter. In all these cases, transcription of the reporter gene can be tracked and therefore gene expression can be studied. Most conventional reporter genes include the bacterial gene chloramphenicol acetyl transferase (CAT), lacZ gene, alkaline phosphatase (ALP), Bla/-lactamase. Autoradiography of a chromatogram (when using CAT), enzyme assay (when using lacZ) or immunohistochemistry (when using CAT or lacZ) can then be used to assay cell extracts for the product of the reporter gene (4). A reporter gene such as ALP which can lead to a protein product secreted into the blood stream can also be used, thereby allowing monitoring in living animals. However, the location(s) of the reporter gene are not able to be determined in this case, because only the blood can easily be sampled.

Other conventional reporter genes such as green fluorescence protein (GFP)[5] and luciferase[6] whose products directly interact with light, allow for localization in some living animals. Although small animals or animals transparent to light can be imaged with cooled charged coupled device (CCD) detector, these imaging techniques are very limited because of their lack of generalizability and detailed tomographic resolution[6]. PET imaging techniques offer the possibility of monitoring the location, magnitude, and persistence of reporter gene expression with a very high sensitivity for in vivo use in animal and humans.

Characteristics of the ideal reporter gene/probe

In theory, the ideal reporter gene/probe would have the following characteristics[7]:

- The reporter gene should be present (but not expressed) in mammalian cells in order to prevent an immune response.
- The specific reporter probe should accumulate only where reporter gene is expressed.
- No reporter probe should accumulate when the reporter gene is not being expressed.
- The product of the reporter gene should also be nonimmunogenic.
- The reporter probe should be stable in vivo and not be metabolized before reaching its target.
- The reporter probe should clear rapidly from circulation and not interfere with detection of specific signal. Neither ther reporter probe nor its metabolites should be cytotoxic.
- The size of the reporter gene with its driving promoter should be small enough to fit into a delivery vehicle (e.g., plasmids, viruses), except that this is not required for transgenic applications.
- The reporter probe should not be prevented by natural biologic barriers from reaching its destination.
- The image signal should correlate well with levels of reporter gene mRNA and protein in vivo.

At present, no single reporter gene/probe system satisfies all these criteria. Therefore, the consequent

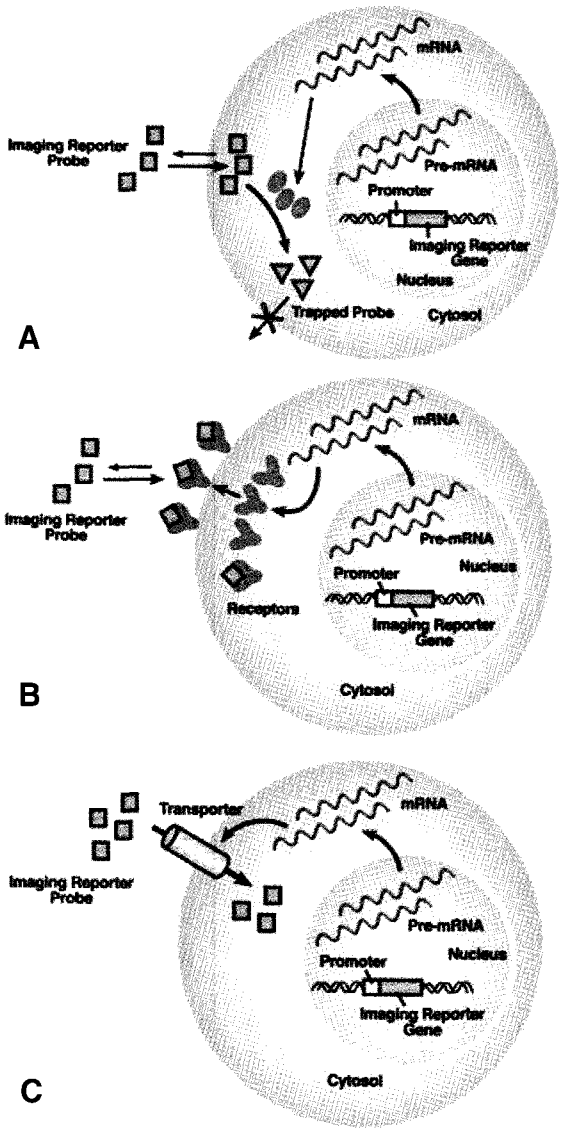

Fig. 1. Three different types of PET imaging reporter gene/probe strategies. (A) Enzyme-based reporter gene imaging system: Radiotracer such as ^{18}F-FHBG is substrate molecules phosphorylated by the imaging reporter enzyme such as the HSV1-tk to result in intracellular trapping of the probe in cells expressing the imaging reporter gene. (B) Receptor-based reporter gene imaging system: Radiotracers such as ^{18}F-FESP or radiolabeled somatostatin analogues are ligand molecular probes interacting with the expressed receptor such as dopamine-2-receptor (D2R) or somatostatin receptor (SSTR2) to result in trapping of the probe on/in cells expressing the D2R gene or SSTR2 gene. (C) Transporter-based reporter gene imaging system: Overexpression of transporter such as sodium iodide symporter (NIS) results in cell uptake of the reporter probe such as radioiodide than would occur otherwise.

development of multiple system provides a choice based on the application area of interest. Additionally, the availability of multiple reporter gene/reporter probes facilitates monitoring the exression of more than one reporter gene in the same living animal.

Classification of Imaging Reporter Gene Systems

A broad classification of reporter systems consists of those where the gene product is intracellular (Fig. 1A), or is associated with the cell membrane (Fig. 1B, 1C). Examples of intracellular reporters include thymidine kinase,[2,8] cytosine deaminase[9,10] to name a few. Examples of reporters on or in the cell surface in the form of receptors include the dopamine 2 receptor (D2R),[2,11] and receptors for somatostatin,[12] or the sodium iodide symporter.[13,14] The major advantages of intracellular protein expression are the relatively uncomplicated expression strategy and lack of recognition of the expression product by the immune system. The major advantages of surface-expressed receptors and acceptors are favorable kinetics (sometimes avoiding the need for the tracer to penetrate into a cell) and the fact that synthetic reporters can be engineered to recognize already approved imaging drugs.[15]

Herpes simplex virus thymidine kinase (HSV1-TK)

Wild type Herpes Simplex Virus Type 1 thymidine kinase *(HSV1-tk)* and a mutant HSV1-tk gene, *HSV1-sr39tk* are the most common reporter genes used in current molecular imaging studies using radiolabeled substrate and PET imaging. *The HSV1-tk* and *HSV1-sr39tk* gene products are enzymes that have less substrate specificity than mammalian thymidine kinase 1 (TK1) and can phosphorylate a wider range of substrate analogues. Substrates that have been studied to date as PET reporter probes for HSV1-TK can be classified into two main categories (Fig. 2) - pyrimidine nucleoside derivatives (e.g. FIAU: 5-iodo-2'-fluoro-2'deoxy-1--D-arabino-furanosyl-uracil),[16-20] and acycloguanosine derivatives (e.g. FPCV: fluoropenciclovir, FHBG: 9-[4-fluoro-3-(hydrommethyl) butyl]guanine),[21-26] and have been investigated in terms of sensitivity and specificity. These radiolabeled reporter probes are transported into cells, and are trapped as a result of phosphorylation by HSV1-TK. When used in non-pharmacological tracer doses, these substrates can serve as PET or SPECT targeted reporter probes by their accumulation in just the cells expressing the *HSV1-tk*

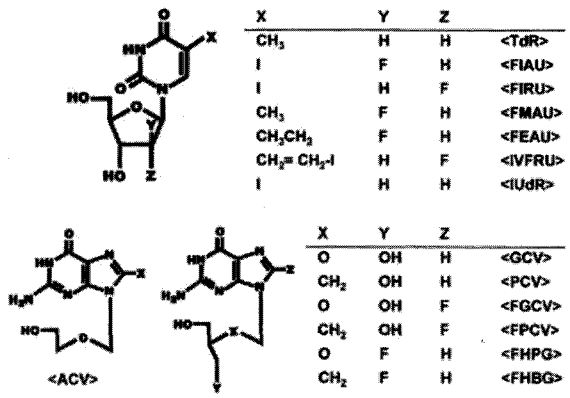

Fig. 2. Structures of thymidine and HSV1-TK reporter gene substrates.
TdR (thymidine), 5-methyluracil-2'-deoxyribose;
FIAU, 2'-fluoro-2'-deoxy-b-D-arabinofuranosyl-5-iodouracil;
FIRU, 2'-fluoro-2'-deoxy-5-iodo-1-b-D-ribofuranosyl-uracil;
FMAU, 2'-fluoro-2'-deoxy-5-methyl-1-b-D-arabinofuranosyl-uracil;
FEAU, 2'-fluoro-2'-deoxyarabinofuranosyl-5-ethyluracil;
IVFRU, 2'-fluoro-2'-deoxy-5-iodovinyl-1-b-ribofuranosyl-uracil;
IUdR, 2'-deoxy-5-iodo-1-b-D-ribofuranosyl-uracil;
ACV, 9-((2-hydroxy-1-ethoxy)methyl)guanine (acyclovir);
GCV, 9-((2-hydroxy-1-(hydroxymethyl)ethoxy)methyl)guanine (ganciclovir);
PCV, 9-(4-hydroxy-3-(hydroxymethyl)butyl)guanine (penciclovir);
FGCV, 8-fluoro-9-((2-hydroxy-1-(hydroxymethyl)ethoxy)methyl)guanine (fluoroganciclovir);
FPCV, 8-fluoro-9-(4-hydroxy-3-(hydroxymethyl)butyl)guanine (fluoropenciclovir);
FHPG, 9-((3-fluoro-1-hydroxy-2-propoxy)methyl)guanine;
FHBG, 9-(4-fluoro-3-hydroxymethylbutyl)guanine.

gene. According to the attempts to improve the sensitivity by using a mutant version of this gene, *HSV1-sr39tk* was derived using site-directed mutagenesis to obtain an enzyme more effective at phosphorylating ganciclovir (GCV) (and also less efficient at phosphorylating thymidine) with consequent gain in imaging signal.[23] Recently, it is reported that the *HSV1-sr39tk* reporter gene system with the ^{18}F-PCV or ^{18}F-FHBG is a better combination over the *HSV1-tk* reporter gene system with FIAU in C6 cell mouse xenografts.

HSV-tk and *HSV1-sr39tk* are nonhuman genes and pose a small risk of generating an immune response against cells and tissue transduced with these genes. One approach to reduce this risk is to use human thymidine kinase 2 (hTK2) gene. The hTK2 gene is minimally expressed in the mitochondria of most human tissues and can be used as a human reporter gene. The hTK2 enzyme has a spectrum of substrate specificity similar to that of viral thymidine kinase. The substrate specificity is broader and less restricted in comparison to that of human thymidine kinase 1 (hTK1), and hTK2 has been shown to phosphorylate radiolabeled FIAU and FEAU (5-ethyl-2'-fluoro-2'deoxy-1--D-arabino-furanosyl-uracil). Therefore, radiolabeled FIAU or FEAU can be administered to patients, and hTK2 may provide an alternative human thymidine kinase reporter system for use in human subjects.[8]

Human dopamine 2 receptor (hD2R)

The dopamine 2 receptor *(hD2R)* reporter gene has also been validated for PET reporter on or in the cell surface in the form of receptors while using ^{18}F labeled fluoroethylspiperone (FESP) as the reporter probe ligand.[27] The advantages of this system is that the expression of hD2R is largely limited to the striatal-nigral system of the brain, and that an established radiolabeled probe, FESP has been extensively used to image striatal-nigral D2 receptors in human subjects.[28]

Human somatostatin receptor subtype-2 (hSSTR2)

Somatostatin receptor imaging has been developed to demonstrate efficacy for detection of naturally SSTR-positive neuroendocrine and lung cancers.[29] Similar to D2R system, the hSSTR2 gene has been suggested as a potential reporter gene for human studies,[30,31] because the expression of hSSTR2 gene is largely limited to carcinoid tumors. Octreotide, P829, and P2045 are synthetic somatostatin analogues that preferentially bind with high affinity to somatostatin receptor subtypes 2, 3, and 5 of human, mouse, or rat origin.[12] Of further significance, somatostatin analogue PET tracers are available with potential for imaging applications using the hSSTR2 reporter gene.[32-34]

Human sodium iodide symporter (hNIS)

Sodium iodide symporter (NIS) is expressed primarily on the basolateral membrane of thyroid epithelial cells. It is responsible for active iodide uptake in thyrocytes, the first essential step in a series of biochemical changes culminating in the incorporation of the ion within tyrosine residues in thyroglobulin, the precursor for thyroid hormone biosynthesis.[14] NIS is also expressed at lower levels, in many other organs such as the salivary and lacrimal

glands, stomach, choroid plexus, lactating mammary gland, kidney epithelial cells and placenta. Ion binding to NIS is non-random; two sodium ions bind first, followed by an iodide ion. NIS can transport into cells many other anions coupled with sodium transport. These include ClO_3^-, SCN^-, $SeCN^-$, NO_3^-, Br^-, TcO_4^-, RhO_4^- and ^{211}At.[35-37]

Groot-Wassink et al.[38] have utilized NIS in combination with ^{124}I to monitor the biodistribution and expression of a replication incompetent adenovirus using PET imaging. ^{124}I is not an ideal radiotracer due to its low positron abundance (25%) and the concomitant emission of high-energy gamma photons that make accurate dosimetric calculations difficult.[39] However, its long half-life (4.12 days) allows tracking of slow biochemical processes and a cyclotron need not be on site for synthesis of the isotope. Gamma camera imaging has provided adequate data for monitoring and quantification of in vivo gene expression as well as dosimetric calculations.

NIS has several potential advantages over the other reporter systems; (1) NIS is a physiologically expressed protein that only rarely induces the immune reaction. (2) The radiotracers used combination with NIS are readily commercially available at a low cost and are already approved by the Food and Drug Administration for clinical applications.

Four categories of applications for PET molecular imaging will be reviewed: imaging of cell trafficking, imaging of immunotherapies, imaging of gene therapies, and imaging of molecular interactions such as protein-protein interactions.

Translational Approaches of Reporter Gene Imaging

Imaging Cell Trafficking

Immune cell or cancer cell trafficking

An important application of PET and reporter constructs is to noninvasively monitor cell trafficking. Imaging can be used to look at different properties of cellular trafficking including metastasis, stem cell transplantation, and lymphocyte response to inflammation. PET radiotracers and reporter genes have been used to study cell migration and anti-tumor responses. ^{18}F-FDG, a common PET tracer used to look at cellular metabolism of glucose as a good indicator of neoplasia, is highly retained in lymphocytes. This aspect of FDG has been exploited to follow monocyte trafficking.[40] Botti et al.[41] compared FDG, as well as ^{99m}Tc-hexamethazine and ^{111}In-oxine labeling in activated lymphocytes to determine which method would be best for tracking adoptively transferred T lymphocytes in patients. ^{64}Cu-pyruvaldehyde-bis(N^4-methylthiosemicarbazone) (Cu-PTSM), has also been assessed in a glioma cell line and in splenocytes.[42] The cells were labeled *ex vivo* with tracer and subsequently injected and monitored in the living animal with microPET. Le et al.[43] assessed the role of the BCR-ABL oncogene and G2A (G protein-coupled receptor (GPCR) predominantly expressed in lymphocytes) in lymphoid leukemogenesis using bone marrow cells marked with *HSV1-tk*. By adapting murine transplantation models of BCR-ABL induced leukemia for micro PET imaging, they revealed G2A functions as a negative modifier of BCR-ABL induced leukemogenesis, and have developed system with the potential to study preleukemic events and candidate cellular/biological processes. Utilizing HSV1-sr39tk as a reporter gene in adoptively transferred lymphocytes, Dubey *et al.*[44] were able to show that T cell anti-tumor responses could be quantified using microPET. Similarly, Koehne *et al.*[45] showed that Epstein-Barr virus (EBV)- specific T lymphocytes, marked with HSV1-tk, can be shown to traffic and accumulate in EBV+ tumors in mice using microPET. These approaches 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. Monitoring cell trafficking is feasible in many disease conditions, although careful consideration must be taken in choosing the labeling agent (possible toxicity) and/or the reporter gene (possible immunogenicity). These types of studies would allow for better understanding of the disease process, and the response of neoplastic and immune cells to therapeutics.

Stem cell trafficking

Because of the capacity for indefinite reproduction, together with the ability to produce differentiated progeny, stem cell transplantation holds potential promise for

treating a wide variety of human diseases, including Parkinson's disease, Alzheimer's disease, stroke, heart disease and joint diseases. The mechanisms may be related to stem cells secreting multiple arteriogenic cytokines, providing mechanical scaffold, or recruiting other beneficial cells to the diseased territory. However, most techniques used for the analysis of stem cell survival in animal models has relied on postmortem histology to determine the fate and migratory behavior of the stem cells. This approach, however, precludes any sort of longitudinal monitoring. An approach which would allow for the monitoring of stem cell activities within the context of the intact whole-body system, rather than with histological slides, would allow us to gain further insights into the underlying biological and physiological properties of stem cells.

Radionuclide Imaging

Several imaging strategies are currently under active investigation, including radionuclide labeling, ferromagnetic labeling, and reporter gene labeling.[46,47] In a study of radionuclide labeling, Aicher *et al.*[48] injected indium-111 oxine-labeled endothelial progenitor cells into the infarcted myocardia of nude rats, and imaged them at 24 to 96 hours, using a gamma camera. The main limitation associated with this approach is that radionuclides have physical half-lives, making it possible to monitor cell distribution only for a limited number of days.

MRI

In a study of ferromagnetic labeling, Kraitchman *et al.*[49] injected swine mesenchymal stem cells (MSCs), which contained ferrumoxide (Feridex) particles, into the hearts of pigs. A 1.5T MRI scanner was used to detect localization of Feridex for up to 1 week, while Amado et al.[50] were able to track the MR signal for 8 weeks in a similar swine model. In a separate study Kraitchman et al.[51] compared the sensitivity of SPECT versus that of MRI in tracking the mobilization of MSCs after intravenous injections into dog with myocardial infarction. By double-labeling MSCs with ^{111}In-oxine and ferumoxides-poly-l-lysine, SPECT was able to detect MSCs in the infracted myocardium within the first 24 hours until 7 days after injection, whereas MRI was unable to detect the small numbers of cells homing in to the site. The advantages of MRI, however, reside in its longer half-lives allowing for repeated imaging over a prolonged study period, its potential to offer high spatial resolution and to translate these techniques into clinical research. However, MRI lacks relative sensitivity and therefore requires a high number of labeled cells to achieve the detection threshold. The major drawback of MRI lies in the uneven distribution of iron oxide within cells, which prevent the accurate correlation of cell number within signal intensity.[46] Furthermore, a dilution of MR probes occurs within each cell division, resulting in weakening the MRI's capability to track cellular proliferation. The most critical problem may be that the viability of labeled cells thus cannot be accurately assessed, as superparamagnetic iron oxide can remain in the cytoplasm even after cell death or can be phagocytosed by surrounding macrophages to register a "false" signal.[52]

Reporter gene imaging

Reporter gene imaging provides several unique advantages over other imaging techniques. First, in contrast to radionuclide- or iron-labeling techniques, only viable cells can be detected because accumulation of reporter probe requires expression of the reporter gene product. Second, the reporter gene can be integrated into the cellular chromosomes and passed on from mother to daughter cells, thereby permitting tracking of cell proliferation. Finally, multiple reporter genes can be combined for multimodality visualization or can be combined with specific promoters for analyses of molecular pathways or differentiation processes in precise manner.[53,54]

During the process of reporter gene labeling, the cells are transfected with reporter genes before being implanted into the myocardium.[55] In cases in which the cells remain alive, the reporter gene will be expressed. In cases in which the cells are dead, the reporter gene will not be expressed. Employing this approach, Wu *et al.*[55] recently used embryonic cardiomyoblasts which express herpes simplex virus type I thymidine kinase (HSV1-tk) or firefly luciferase (Fluc) reporter genes, which they then noninvasively tracked using either micro-PET or bioluminescence optical imaging. Drastic reductions were noted in signal intensity within the first 1 to 4 days, and

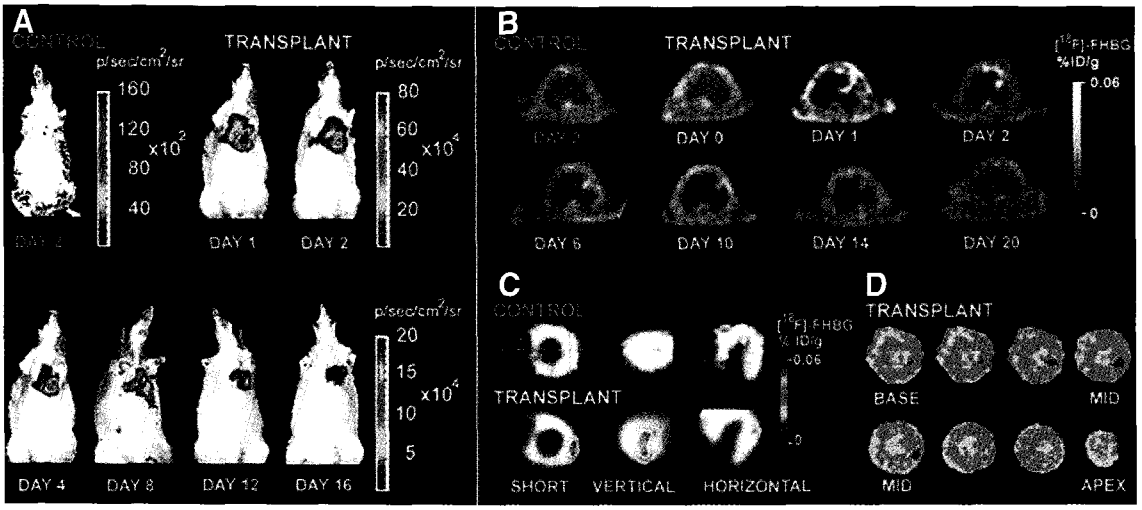

Fig. 3. Reporter gene imaging of cardiac cell transplantation in living animals. (A) Optical imaging shows a representative rat transplanted with embryonic cardiomyoblasts expressing the firefly luciferase reporter gene (Fluc) that emits significant cardiac bioluminescence activity on days 1, 2, 4, 8, 12, and 16 ($P<.05$ vs control). The control rat (injected with cardiomyoblasts transfected with HSV1-sr39tk, which served as a negative control) has background signal only. (B) MicroPET imaging shows longitudinal imaging of ^{18}F-FHBG reporter activity in a representative rat transplanted with cardiomyoblasts expressing the HSV1-sr39tk reporter gene (*gray scale*). (C) Detailed tomographic views of cardiac microPET images are shown in the short, vertical, and horizontal axis. On day 2, a representative transplant rat has significant activity at the lateral wall, as shown by the F-18 FHBG reporter activity image (*color scale*) overlaid on the ^{13}N-ammonia perfusion image (*gray scale*). In contrast, the control rat (injected with cardiomyoblasts transfected with Fluc, which served as a negative control) has homogeneous ^{13}N-ammonia perfusion but background ^{18}F-FHBG reporter activity. (D) Autoradiography of the same study rat confirms trapping of ^{18}F radioactivity by transplanted cells at the lateral wall at a finer spatial resolution (approximately 50 μm). *p/sec/cm²/sr*, Photon/second/centimeter squared/steridian; ID/g, injected dose/gram. (Reproduced and adapted with permission from Wu JC, Chen IY, Sundaresan G, Min JJ, De A, Qiao JH, *et al*. Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. Circulation 2003;108:1302-5.)

this was tentatively attributed to acute donor cell death as the result of inflammation, adenoviral toxicity, ischemia, or apoptosis (Fig. 3).

In a recent study Cao et al[56] conducted the first investigation to study the kinetics of embryonic stem (ES) cell survival, proliferation, and migration after intramyocardial transplantation by using multimodality imaging system. In this study, mouse ES cells carrying a novel triple-fusion (TF) reporter gene consisting of Fluc, monomeric red fluorescence protein, and herpes simplex virus type 1 truncated thymidine kinase (HSV1-ttk) were injected into the myocardium of adult nude rats. By use of bioluminescence and PET imaging system, the survival and proliferation of injected ES cells were followed in vivo for 4 weeks (Fig. 4). Postmortem analysis revealed teratoma formation by week 4. Interestingly, HSV1-ttk, besides being a PET reporter gene, can also be used as a suicide gene by administering pharmacologic dosages of ganciclovir, which can terminate deoxyribonucleic acid synthesis in cells that carry the viral HSV-ttk. Intraperitoneal injection of ganciclovir (50 mg/kg twice daily) starting at week 3 after cell transplantation successfully ablated teratomas in the treated group, as demonstrated by the disappearance of PET and bioluminescence signals.

One potential concern regarding reporter gene is that they may exert adverse effects on stem cell biology and function. To address this issue, Wu et al[57] recently compared gene expression profiling in ES cells expressing the TF reporter gene with normal ES cells. Microarray analysis revealed only a small percentage of genome (1-2%) that underwent transcriptional changes in ES cells with TF. Out of 20,371 genes studied, 173 were downregulated and 123 were upregulated. The downregulated genes were involved with cell cycle regulation, cell death, and nucleotide metabolism, whereas the upregulated genes were involved in antiapoptosis and homeostasis pathways. However, despite transcriptional changes resulting from the introduction of exogenous TF reporter genes, there appear to be no significant effects on ES cell

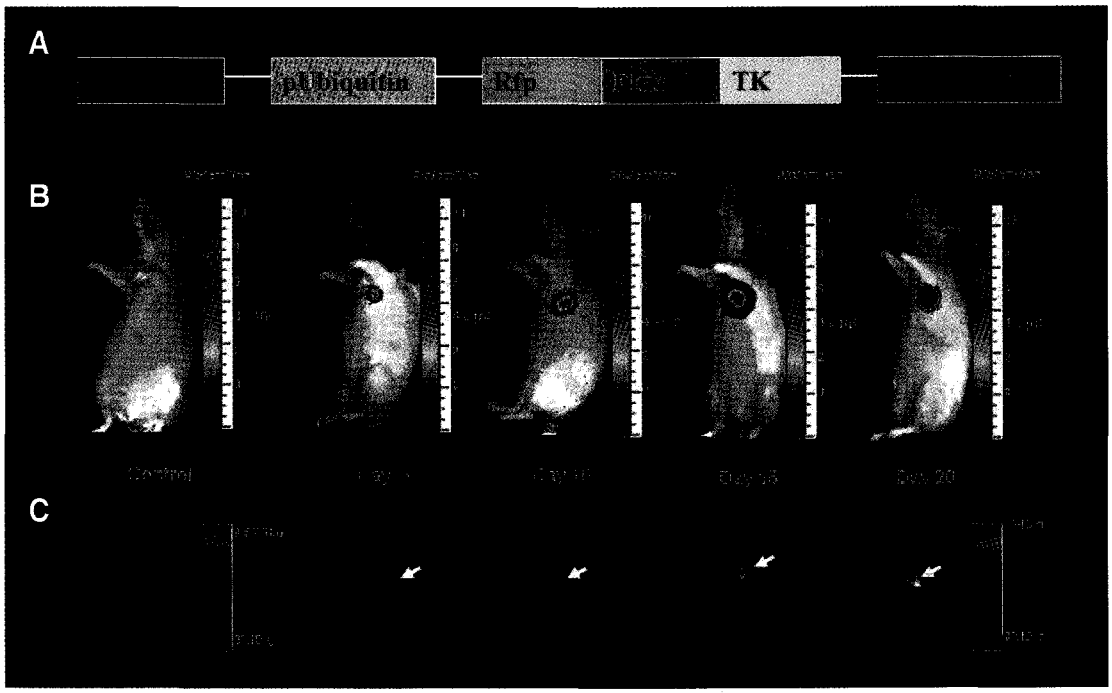

Fig. 4. Multimodality molecular imaging of embryonic stem cells in living animals. (A) Schema of the *TF* reporter gene containing fusion of Fluc, monomeric red fluorescence protein (mrfp), and HSV1-ttk (ttk). (B) Optical bioluminescence imaging showing mouse ES cells expressing the Fluc gene on day 4 and at week 1, week 2, week 3, and week 4 after intramyocardial transplantation. (C) Detailed tomographic view of cardiac PET imaging of ^{18}F-FHBG activity showing mouse ES cells expressing the HSV1-ttk reporter gene at corresponding time points. *LVLTR*, Lentivirus long terminal repeats; *pUbiquitin*, ubiquitin promoter; *SIN*, self-inactivating. (Reproduced and adapted with permission from Cao F, Lin S, Xie X, Ray P, Patel M, Zhang X, et al. In vivo visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. Circulation 2006;113:1005-14.)

viability, proliferation, and differentiation pathways.[56,57] Thus, in light of its unique abilities to image cells longitudinally and noninvasively, multimodality molecular/reporter gene imaging holds tremendous promise as a tool to track the fate of stem cells in stem cell therapy.

Imaging Gene Therapies

Monitoring of in vivo gene expression is critical for the evaluation of the success or failure of the gene therapy approaches. Tissue biopsy might provide some insight into this issue but some organs are not accessible and repeated biopsies cause inconvenient stress. Thus, imaging techniques for noninvasive and longitudinal monitoring of therapeutic gene expression are highly desirable. Previously, to assay the expression of a therapeutic gene, invasive techniques were used, but reporter genes have been validated that can be used in PET imaging, to study gene expression *in vivo*.

The reporter gene can itself be the therapeutic gene or can be coupled to the therapeutic gene (2). 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 GCV can be coupled with imaging of the accumulation of radiolabeled probes (^{18}F-FHBG or ^{124}I-FIAU). Jacobs et al.[58] 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. This study showed that [^{124}I]FIAU PET is feasible and that vector-mediated gene expression may predict a therapeutic effect. Other reporters, sodium/iodide symporter (NIS) which facilitate the uptake of iodide by thyroid follicular cells is also being applied in radioiodide gene therapy.[13] The conventional radioiodide or ^{99m}Tc-pertechnetate scintigraphy has been used to directly monitor NIS expression. Since the iodine is not trapped, issue of efflux have to be optimized but initial studies show significant promise. Human somatostatin subtype 2 receptor

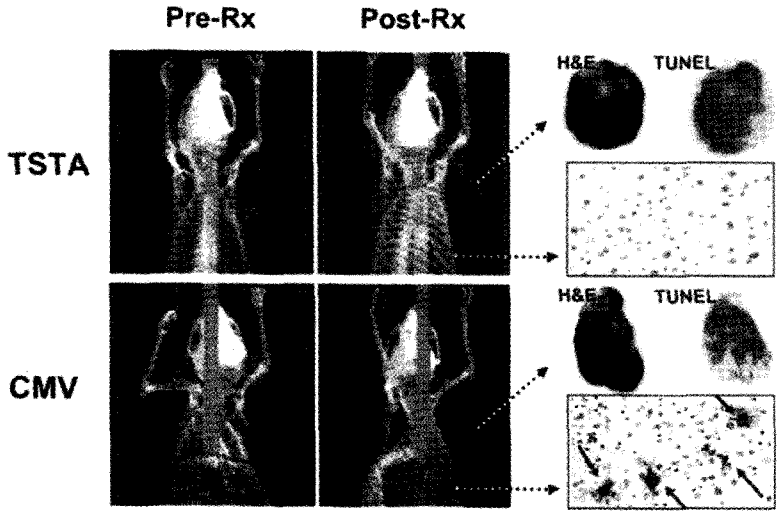

Fig. 5. MicroPET/CT imaging of suicide gene therapy. Ten to the ninth infectious units of prostate-targeted AdTSTA-sr39tk (TSTA) or constitutive active AdCMV-sr39tk (CMV) was intratumorally injected into androgen dependent LAPC-4 tumors, human prostate cancer xenografts on mice on day 0. MicroPET/CT imaging performed prior to ganciclovir (GCV) treatment on day 7 showed tumor-limited expression in the TSTA-treated animals, but the CMV-treated animal showed strong expression in the liver as well. After receiving GCV treatment, F18-FHBG PET signals at day 22 were diminished in the tumors and the liver of the CMV animals. Histology performed at the end-point (day 22) revealed extensive apoptosis (TUNEL-positive brown staining) in the tumors and the liver of the CMV-treated animals. (Reproduced and adapted with permission from Johnson M, Sato M, Burton J, Gambhir SS, Carey M, Wu L. Micro-PET/CT monitoring of herpes thymidine kinase suicide gene therapy in a prostate cancer xenograft: the advantage of a cell-specific transcriptional targeting approach. Mol Imaging 2005;4:463-72.)

(hSSTR2) is also being applied in radiotargeted gene therapy in combination with radiolabeled synthetic somatostatin analogues.[59]

Transcriptional targeting

Transcriptional targeting is feasible because the tissue- or cancer-specific promoter can be activated in the targeted cells in the presence of the proper subset of activators, but would remain relatively silent in the non-targeted cells. A wide range of tissue-specific and tumor-selective promoters (TSPs) has been developed for gene therapy of cancer.[60,61] However, transgene expression involving the use of TSPs is generally lower than constitutive viral promoters due to their weak transcriptional activity.

A two-step transcriptional amplification (TSTA) method for amplifying gene expression using weak promoters has been developed by Iyer et al.[62] In this approach, a specific promoter directed the potent transcription activator, GAL4-VP16, which in turn acted upon a second GAL-4-responsive reporter or therapeutic gene. This approach can enhance the activity of the prostate specific antigen (PSA) promoter over a range of up to 1000-fold.[63,64] Follow-up application has been documented with enhancement of the carcinoembryonic antigen (CEA) promoter to boost HSV1-tk expression. Greater tumor cell killing of CEA positive breast cancer cell and accumulation of ^{131}I-FIAU tumor signal recorded by gamma camera were documented in this study.[65]

The potency and specificity of the PSA promoter-based TSTA expression system was retained in an adenovirus vector.[66,67] In a very recent study,[68] combined ^{18}F-FHBG PET and CT were utilized to monitor intratumoral gene transfer and therapy mediated by the prostate-specific AdTSTA-sr39tk or AdCMV-sr39tk adenoviral vectors (Fig. 5). Loss of FHBG PET signal in the tumor was observed, which correlated with destruction of tumor cells. In this study, tissue specific TSTA suicide gene therapy was demonstrated to be superior to the constitutive approach in minimizing systemic liver toxicity evidenced

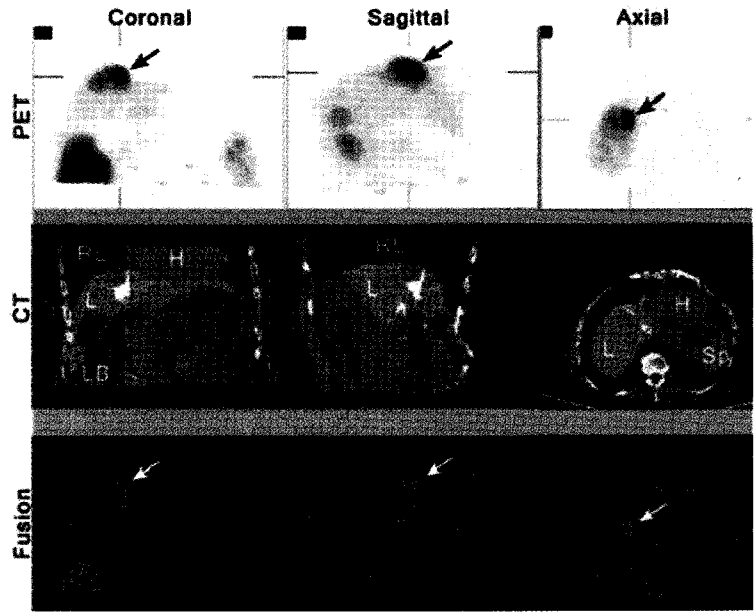

Fig. 6. PET imaging of adenoviral-mediated transgene expression in liver cancer patients. PET-CT imaging of HSV1-tk transgene expression in humans. Columns 1 to 3 show the 5-mm-thick coronal, sagittal, and transaxial slices, respectively, from a (18F)FHBG-PET-CT study in patient 5. All sections are centered 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. (Reproduced and adapted with permission from Penuelas I, Mazzolini G, Boan JF, Sangro B, Marti-Climent J, Ruiz M, et al. Positron emission tomography imaging of adenoviral-mediated transgene expression in liver cancer patients. Gastroenterology. 2005;128:1787-95.)

by no hepatic radioactivity in PET images. A lentivirus carrying the TSTA expression cassette also exhibited regulated, cell-specific and long-term expression.[69] In a separate study,[70] The regulation of GRP78 promoter which regulate the expression of a stress-inducible chaperone protein, GRP78, was examined in human breast tumor by ^{18}F-FHBG. ^{18}F-FHBG PET signals were documented in breast tumors stably transduced by a retrovirus carrying HSV-tk driven by the GRP78 promoter. The signal was more induced by photodynamic therapy.

Linked gene expression strategies

This 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 and optical imaging through a variety of different molecular constructs. Examples include fusion approaches,[71,72] bicistronic approaches using internal ribosomal entry site (IRES),[73,74] dual-promoter approaches,[75,76] a bidirectional transcriptional approach,[77] and a two vector administration approach.[78]

A fusion gene approach can be used in which two or more different genes are joined in such a way that their

coding sequences are in the same reading frame, and thus a single protein with properties of both the original proteins is produced. 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.[79] Another approach is to insert an IRES sequence between the two genes so that they are transcribed into a single mRNA from the same promoter but translated into two separate proteins. 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.[74] 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.[75] 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.[77] 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. 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.

Clinical Applications and Future Prespects

The ultimate objective of reporter gene imaging is to be able to translate the findings from small animal models to clinically applicable methods. In this context, some recent clinical studies have incorporated imaging to monitor cancer gene therapy. For example, repetitive PET imaging was performed to assess a cationic liposome-mediated HSV1-tk suscide gene transfer into glioblastoma.[58,80] Vector-mediated HSV1-tk gene expression was monitored by ^{124}I-FIAU in five patients with recurrent glioblastoma. In one patient, specific ^{124}I-FIAU uptake was detected within the infused tumor. After ganciclovir treatment, signs of necrosis were observed by FDG-PET indicating HSV1-tk mediated treatment response. The use of ^{18}F-FHBG probe was studied in healthy human volunteers to characterize the biodistribution and route of clearance of the reporter probe. The results showed that FHBG exhibited good pharmacokinetic properties and rapid clearance suitable for applications in patients.[81] More interestingly, FHBG-PET has been used to monitor HSV1-tk suscide gene expression in patients with hepatocellular carcinoma.[82,83] Gene expression was evident in all patients who received a viral dose of 7.7×10^9 pfu or more (Fig. 6). These findings will help support the use of the FHBG- HSV1-tk system to directly monitor the expression of therapeutic gene in future gene therapy trials. Currnetly, human trial with T cells and dendritic cells expressing HSV1-tk reporterand consecutive ^{18}F-FHBG PET imaging study has been approved by the the Food and Drug Administration.

Molecular imaging strategies will likely expand significantly over the next few years as imaging and molecular genetic technologies continue 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 investigators may readily move between the various technologies should help to also test various pre-clinical models. This potential power of molecular imaging to see fundamental biological processes in a new light will not only help to enhance our knowledge and understanding but should also accelerate considerably the rate of discovery in the biological sciences.

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