Molecular Imaging of Pulmonary Gene Expression with Positron Emission Tomography

Sekhar Dharmarajan and Daniel P. Schuster

Departments of Surgery, Medicine, and Radiology, Washington University School of Medicine, St. Louis, Missouri

Gene expression imaging is one form of molecular imaging used to visualize, characterize, and quantify, spatially and temporally, normal as well as pathologic processes at cellular and subcellular levels within intact living organisms. Most studies to date have employed positron emission tomography as the imaging platform to detect, monitor, and quantify gene expression in the lungs. These studies have shown that imaging can be used to determine the onset and duration of transgene expression, the effectiveness of different gene delivery systems, and the linearity of vector dose-response relationships. This rapidly developing field can be expected to provide useful new tools with which to study gene expression in transgenic animals and in humans during gene therapy.

Keywords: FHGB; gene reporters; thymidine kinase

Pulmonary scientists are now able to call on a suite of powerful imaging techniques to characterize lung physiology and pathophysiology (Figure 1 [p. 514]). *Anatomic imaging* methods are used to display structure (e.g., airway diameter) or to make measurements related to structure (e.g., lung volumes such as functional residual capacity). *Functional imaging* methods usually depend on obtaining repeated data over time to measure biologic processes such as ventilation, perfusion, or pulmonary artery pressures. Thus, anatomic imaging involves long time constants; changes, if any, in the structure or process being imaged occur over a period of hours, days, or even years. Changes mapped by functional imaging occur more quickly, usually over seconds to hours. Finally, *molecular imaging* methods (1, 2) include techniques that are used to detect the presence or activity of specific molecular targets in tissues of interest (e.g., the expression of transgenes). Images from more than one modality can be fused, allowing structure-function and function-function relationships to be studied on a regional basis.

In addition to dramatic advances in new high-resolution imaging instrumentation that now make studies in small animals possible (Figure 2 [p. 515]) (3), related progress in the development of highly specific probes as sources for imaging contrast, and in molecular and cell biology techniques that can be adapted for *in vivo* imaging studies, have helped spur the rapid expansion of imaging methods to the study of disease biology. These advances are now being exploited to characterize fundamental processes, such as gene expression, inflammation, cell trafficking, and apoptosis. Broad overviews of molecular imaging, including those pertaining specifically to the lungs, can be found elsewhere (1–8). Here, we focus primarily on the use of molecular imaging

with positron emission tomography (PET) to study gene expression in the lungs.

GENE EXPRESSION IMAGING

Classically, monitoring gene expression usually requires tissue sampling to, for instance, measure messenger RNA levels, but such methods are invasive and unattractive as routine procedures for clinical investigators. In contrast, gene expression imaging, at least ideally, can provide a seamless transition from studies in animals to later studies in humans.

Theoretically, gene expression imaging could be implemented at either the transcriptional (messenger RNA) or translational (protein) level. Unfortunately, to date, strategies to image gene expression at the messenger RNA level have been limited by difficulties in delivering appropriate imaging probes to the molecular targets in sufficient quantity, and, because the number of target molecules per cell is often low, extreme amplification of the imaging signal is often required for visualization (9, 10). However, these limitations are being addressed through the use of nanotechnologies to deliver highly specific imaging probes that bind to messenger RNAs that are overexpressed in tumor tissues (11). This strategy has yet to be applied to studies of lung biology.

Overall, however, molecular imaging of gene expression has focused on protein products as targets (e.g., receptors, membrane transporters, or intracellular enzymes) because they are more plentiful within a cell, may be overexpressed during disease, and are often more readily available to molecular probes.

Most *in vivo* molecular imaging studies of gene expression have adapted the *in vitro* strategy of monitoring gene expression by measuring the expression of a suitable reporter gene. In such cases, the imaging signal is derived from the activation or binding of the imaging probe to a reporter protein that is linked to the gene product of interest. The main advantage of this strategy is that only a small number of reporter-probe combinations is needed; rather than developing probes specific for each gene of interest, a few well-characterized reporter genes are used as targets for molecular imaging. These genes can then be linked to the actual genes of interest (e.g., therapeutic or biology-modifying genes), using standard molecular biology techniques. In the end, the assumption is that imaging of reporter gene expression accurately reflects expression of the linked gene of interest.

When tissue-based methods are used to monitor reporter gene expression, tissues must be obtained by biopsy, or the animal must be killed so that the required tissues can be harvested. In contrast, molecular imaging of reporter gene expression has the advantage of (1) allowing repeated assessment of gene expression over time within the same animal, (2) avoiding any perturbations to the underlying tissue, and (3) visualizing the full spatial distribution of gene expression throughout the entire body, as opposed to simply in the sampled tissues (1). Although noninvasive *in vivo* imaging of reporter gene expression in intact organisms is now possible with a variety of imaging platforms (12–16), the rest of this discussion is limited to studies using PET to study gene expression in the lungs.

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Correspondence and requests for reprints should be addressed to Daniel P. Schuster, M.D., University Box 8225, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110. E-mail: daniel.schuster@wustl.edu

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PULMONARY GENE EXPRESSION IMAGING WITH PET

To date, studies of pulmonary gene expression imaging using nuclear imaging platforms have primarily used PET as the detection system of choice. Advances in scintillation detector technology have made possible the development of so-called micro-PET scanners, dedicated to small animal investigation, with a spatial resolution of 2 mm or better (17, 18). These scanners are ideal for molecular gene expression imaging studies in small animals, including genetically modified mice.

PET imaging is based on detecting the tissue concentration of compounds labeled with radioactive isotopes that decay by positron emission (Figure 3 [p. 515]). Emitted positrons quickly interact with ambient electrons, producing an annihilation event that results in the simultaneous emission of two photons of equivalent energy traveling 180° apart from one another. It is these high-energy annihilation photons that are actually detected by scintillation crystals placed around the subject so that the source of photon emission can be localized. The main advantages of PET, especially with respect to pulmonary gene expression imaging, are its high degree of sensitivity (level of detection on the order of 10^{-11} to 10^{-12} M of radiotracer), and isotropism (i.e., ability to detect tracer accurately regardless of tissue depth, unlike light-based techniques, which are largely limited to detection at the body surface). The relatively lower spatial resolution of PET (e.g., especially when compared with X-ray computed tomography or magnetic resonance imaging) is less of an impediment when it is the lungs that are to be imaged, compared with organs such as the brain or heart, where limitations on spatial resolution could result in areas being imaged with entirely different structures or functions.

For PET imaging of gene expression, a reporter transgene (PET reporter gene [PRG]) whose protein product is capable of trapping or binding a suitable positron-emitting radiotracer (PET reporter probe [PRP]) is introduced into tissues of interest by any one of a number of methods (Figure 4 [p. 515]). The imaging signal is generated as the PRP accumulates over time only in cells or tissues that express the PRG.

The first demonstration of this concept was reported in an *in vivo* animal tumor model by Tjuvajev and coworkers (19). Since then, a number of different PRP-PRG combinations have been developed and studied, with the PRG encoding exogenous enzymes or cell membrane transporters capable of trapping the PRP in transduced cells, or with the PRG encoding membrane-bound receptors that bind the PRP (20–24). Of these, enzyme-based methods are especially attractive because they can amplify the imaging signal as each reporter protein can metabolize and trap multiple probe molecules. Alternatively, receptor-based techniques have the advantage of not requiring possibly rate-limited transport of the probe into the cell interior in order to be trapped (as in enzyme-based methods). These issues are directly relevant to pulmonary studies of gene expression imaging (see below).

Strategies for introducing reporter genes into living tissues include vectors of various sorts, including viral as well as nonviral (e.g., liposomal) examples. To date, almost all studies of reporter gene expression imaging in the lungs have used adenoviral vectors carrying mutant variants of the murine herpes simplex virus type 1 thymidine kinase gene (mHSV1-*tk*) as the PRG and the nucleoside 9-(4-[¹⁸F]fluoro-3-hydroxymethylbutyl)guanine ([¹⁸F]FHBG) as the PRP (28–32).

For lung studies, the vectors can be delivered either intravenously or intratracheally. Adenovectors that are given intravenously tend to be taken up primarily by the liver, yielding little gene transfer into the lungs (or other organs). Reynolds and coworkers have reported the use of transductional retargeting

(using bispecific antibodies with high affinity for lung targets) and transcriptional enhancements (using tissue-specific promoters) to increase the lung expression of imaging genes (25).

Most studies, however, have avoided these issues by direct intratracheal delivery of the vector. An example is shown in Figure 5 (p. 516), in which a fusion gene of mHSV1-*tk* and enhanced green fluorescent protein (*egfp*), driven by the constitutive cytomegalovirus promoter, is administered intratracheally via a replication-deficient adenovirus. At various times after administration of the virus, expression of the reporter *tk* gene can be assessed by intravenous administration of [¹⁸F]FHBG, a substrate for the thymidine kinase protein. Only tissues expressing the viral *tk* gene will trap this radiopharmaceutical and generate an imaging signal, which can be detected with an appropriate PET scanner (Figure 4 [p. 515]). In this example, the adenovectors were delivered intratracheally in a surfactant vehicle, which has been shown (by imaging) to yield higher levels of gene transfer than a saline vehicle (26).

In an initial study, the ability of PET to image pulmonary transgene expression in normal murine lungs was assessed (27). The amount of radioactivity in the lungs, quantified from the PET images, correlated strongly and linearly with an *ex vivo* assessment of pulmonary radiotracer uptake as measured by gamma counter radioactivity. This observation established that the imaging instrumentation and reconstruction yielded accurate measurements of lung tissue radioactivity, even in murine lungs, a critical criterion for using these measurements to assess gene expression.

An interesting observation from this study was that it was possible to detect transgene expression (i.e., radiotracer accumulation in the lungs) at low viral doses that produced little to no quantifiable transgene expression as measured by standard *in vitro* assays (e.g., fluorescence microscopy for green fluorescent protein)—a testament to the exquisite sensitivity of PET.

In a subsequent study, these methods were used to show that imaging can be used to study the onset and duration of transgene expression (28), an issue of importance both for gene therapeutics and for studies that seek to determine relationships between the effectiveness of gene transfer and the biological effects of the gene product. Significant uptake of the radiotracer could be detected in the lungs as early as 4 to 6 h after gene transfer, with peak levels obtained 4 d after adenovector delivery. Results were identical in animals that were studied once and in those studied repetitively at each time point, illustrating that imaging methods can be used to monitor gene expression efficiently—an advantage when using genetically altered animals in short supply.

Because the lungs are large organs, and because disease within the lungs can be spatially heterogeneous, methods to evaluate gene expression in the lungs should be able to monitor potentially important spatial differences in expression. Imaging methods represent an ideal response to this need. For instance, in a study that assessed differences in the effectiveness of gene transfer when the adenovector was delivered in a surfactant or in a saline vehicle (26), we found that the uptake of the radiotracer [¹⁸F]FHBG was more peripheral and more homogeneous in animals administered the adenovector in the surfactant vehicle. However, the regions of increased tracer concentration (and thus, presumably, increased reporter gene expression) were evenly distributed throughout the lungs (Figure 6 [p. 516]).

The use of reporter gene expression imaging will find its greatest use when studying actual lung disease. Therefore, it is important to demonstrate that imaging strategies would not be confounded by PRG or PRP delivery to edematous or inflamed lung. Accordingly, we undertook a study to assess the ability of PET to characterize transgene expression in a rodent model of

lung transplantation, with acute lung injury induced by ischemia–reperfusion injury or acute rejection (Figure 1 [p. 514]) (29). In this study, there was no loss of gene expression in transplanted and injured lungs, as measured by reporter imaging, compared with expression levels in normal lungs.

As previously mentioned, the use of a reporter gene strategy to monitor gene expression depends on the assumption that the reporter faithfully represents expression of the target (therapeutic) gene in the presence of disease as well as in normal tissues. However, in our first study, we found that peak levels of gene expression on Day 4 increased by a factor of two to three over baseline measurements as assessed by PET, whereas *in vitro* assays indicated a 50- to 150-fold increase in gene expression. This observation suggested that PET imaging of this particular PRG–PRP combination may not fully track the magnitude of gene expression, especially in tissues with high levels of expression. However, we have found that whereas the correlation between PET signal intensity and tissue tracer uptake is strong, the correlation between tissue tracer uptake and tissue-based assays of *tk* as the reporter gene has been modest at best (27, 30). The strength of the correlation between [¹⁸F]FHBG uptake and *in vitro* assays of *tk* expression in other tissues has been variable, with some reports showing a relatively poor correlation, as in our studies, and others showing a stronger correlation (19, 31, 32). Of interest, when pulmonary vascular permeability was increased by pharmacologic means, we were able to obtain a better correlation between imaging and *in vitro* assays of gene expression (30). We interpreted these findings as suggesting that the pulmonary endothelium and interstitial tissues present a significant barrier to the uptake of intravenously delivered PET reporter probes by airspace epithelial cells expressing the PET reporter gene. Accordingly, in normal rat lungs, uptake of the reporter probe appears to be a function of both transport into tissues expressing the transgene and the level of transgene expression itself. These difficulties highlight the fact that the PRP–PRG used in these studies should be viewed as a “first-generation” system, one that will undoubtedly improve as alternative reporter genes, probes, and instrumentation are developed.

In the lungs, some alternative strategies that might improve the correlation between the imaging signal and assays of gene expression based on the direct measurement of gene transcription (e.g., messenger RNA levels) or on measurements of the gene product (e.g., enzyme activity) are already being explored. These include (1) “transductional” retargeting of adenovirus vectors to the lungs so that the transgenes can be delivered via the vasculature, as previously mentioned (25), (2) the use of liposomes rather than adenovirus as the delivery vehicle for the reporter transgene (thereby permitting more effective transfer of intravenously administered transgenes) (32, 33), and (3) alternative combinations of imaging reporter genes and reporter probes (34).

CONCLUSIONS

It is certainly possible, if not likely, that the evaluation of gene therapy for diseases such as cystic fibrosis or lung cancer will be one of the first clinical applications for gene expression imaging (35, 36). The detection of effective gene transfer and expression in patients has been a significant limitation to the evaluation of gene therapy for pulmonary disease, and reporter gene imaging provides a promising noninvasive solution to this problem. Furthermore, transient transgene expression is a problem that plagues the clinical application of many gene therapy protocols, and again, reporter gene imaging provides a noninvasive means for assessing the duration of transgene expression and the need, if any, for repeat therapeutic gene administration. In

another application, pulmonary transcriptional expression of an endogenous gene in transgenic animals can be monitored by using the corresponding promoter/enhancer of the endogenous gene to drive expression of the reporter gene (37, 38). Despite issues raised regarding quantitation of reporter gene expression in the lungs by imaging methods, as discussed above, it already seems clear that these methods can provide useful information about the onset and duration of transgene expression in the lungs, about the effectiveness of different gene delivery systems, and about vector–dose relationships. It is reasonable to expect that gene expression imaging will eventually help hasten the translation of gene therapy protocols to the clinical arena.

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