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PET imaging of gene expression

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Abstract

Noninvasive *in vivo* molecular imaging has developed over the past decade and involves nuclear (Positron emission tomography (PET), gamma camera), magnetic resonance, and *in vivo* optical imaging systems. Most current *in vivo* molecular imaging strategies are ''indirect'' and involve the coupling of a ''reporter gene'' with a complimentary ''reporter probe''. Imaging the level of probe accumulation provides indirect information related to the level of reporter gene expression. Reporter gene constructs are driven by upstream promoter/enhancer elements; they can be constitutive leading to continuous transcription and used to identify the site of transduction and to monitor the level and duration of gene (vector) activity. Alternatively, they can be inducible leading to controlled gene expression, or they can function as a sensor element to monitor the level of endogenous promoters and transcription factors. Several examples of imaging endogenous biological processes in animals using reporter constructs, radiolabelled probes and PET imaging are reviewed (p53-dependent gene expression and T-cell receptor-dependent activation of T-lymphocytes). Issues related to the translation of non-invasive molecular imaging technology into the clinic are discussed.

Keywords: Molecular imaging; Gene imaging; PET; Gamma camera; FIAU; Herpes virus type one thymidine kinase; *HSV1-tk*; p53; T-cell activation; NFAT

1. Introduction

Positron emission tomography (PET) is an established imaging modality that has evolved over the past 30+ years and is now widely used in the clinic, particularly in oncology to define the extent of disease (for staging prior to more invasive procedures) and to identify recurrent disease. An advantage of PET imaging is the ability to obtain specific information about physiological, biochemical and molecular processes in the body. This information is quantitative (based on radiotracer principles), can be presented in three-dimensional space, and can be obtained repeatedly (sequentially) over time in the same subject. A more detailed description of PET imaging technology has been provided in previous chapters. A point that will be emphasised in this chapter is that PET is well suited to image the expression of ''marker''/''reporter'' transgenes. Recent studies have shown that it is possible to image endogenous molecular events using PET. This advance has been largely due to the development of a new class of reporter constructs, complimentary radiolabelled molecules (probes) and novel imaging paradigms.

''Molecular imaging'' is a term that was developed in the 1990s, with roots that go back to in situ visualization of target molecules and biological processes (in situ optical imaging) [1–4]. Molecular imaging has evolved and become a more broadly defined term over the past decade; it includes studies that were previously described as ''gene imaging'' and now relates to many aspects of biology. Advances over the past 5–10 years have included non-invasive *in vivo* molecular imaging in animals. Although it may appear somewhat presumptuous to imply that current non-invasive imaging technologies

Abbreviations: PET, positron emission tomography; FDG, fluorodeoxyglucose; HSV1-tk, herpes simplex virus type 1 thymidine kinase; FIAU, 5-iodo-2′-fluoro-2′deoxy-1-β-D-arabino-furanosyl-uracil; FHBG, 9-(4-fluoro-3-hydroxymethylbutyl)guanine; ACV, acyclovir; GCV, ganciclovir; RASONs, radiolabelled small oligonucleotides; hD2R, human dopamine 2 receptor; FESP, (fluoroethyl)spiperone; hSSTR2, human somatostatin receptor subtype-2; IRES, internal ribosome entry site; eGFP, enhanced green fluorescent protein; TCR, T-cell receptor; NFAT, nuclear factor of activated T-cells; IRES, internal ribosome entry site.

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(magnetic resonance, PET, gamma camera, etc.) can image molecular events that occur within cells, it has already been shown that it is possible to image transcriptional regulation of endogenous gene expression [5]. Needless to say, current PET, gamma camera, magnetic resonance and optical technologies that are used to image animals and patients do not visualise individual cells, much less molecules. What is so exciting about this emerging new field relates to the novel imaging paradigms that are being developed. These paradigms can be successful within the inherent spatial resolution limits of existing imaging systems, because some degree of tissue (cell) homogeneity within the resolution elements (pixels) of the resultant images can be achieved.

2. Imaging strategies

Two molecular imaging strategies—"direct" and "indirect"— will be described, and examples of each will be discussed. "Direct" imaging of endogenous genes and molecules can be defined in terms of a probe-target interaction, whereby the resultant image of probe localisation and magnitude (image intensity) is directly related to its interaction with the target molecule, epitope or enzyme. Indirect molecular-gene imaging is a little more complex in that it may involve multiple components. One example of indirect imaging that is now being widely used is "reporter imaging". This indirect imaging paradigm usually includes a "marker/reporter gene", a "marker/reporter probe" and imaging technology that can visualise the spatial distribution of a probe (that identifies the site of reporter gene expression). In the case of PET, quantitative information about the level of reporter gene expression can also be obtained. Examples of direct and indirect imaging using radiolabelled probes and PET to visualise endogenous molecular processes, such as the regulation of endogenous gene expression, will be discussed below.

2.1. Direct imaging

PET imaging of gene expression using a "direct" approach can be accomplished by developing radiolabelled probes that bind selectively to highly specific regions of the target molecule (e.g. protein, mRNA or DNA), or radiolabelled probes that are selectively metabolised by a specific enzyme or series of reactions that results in a radiolabelled product that is "trapped" in the tissue. Examples of the direct imaging paradigm are common in nuclear medicine. PET imaging of receptor density/occupancy using small radiolabelled molecular probes has been widely used in various fields, particularly in neuroscience research. Imaging cell surface-specific antigens or epitopes with radiolabelled antibodies is another example of direct molecular imaging that has developed over the past 30 years. These studies represent some of the first "molecular imaging" applications and are widely used in clinical nuclear medicine research.

More recently, the visualisation of various tumour cell surface receptors using radiolabelled regulatory peptides is increasingly being pursued in oncology research. These regulatory peptides are small, readily diffusible, and potent natural substances with a wide spectrum of receptor-mediated actions. High affinity receptors for these peptides are frequently overexpressed in many cancers and represent molecular targets for cancer diagnosis and therapy. A recent review summarises peptide-based radiopharmaceuticals, which are presently commercially available or are in advanced stages of clinical testing [6].

Similarly, radiolabelled oligonucleotide antisense and aptomer probes have been developed to specifically hybridise with target mRNA or proteins for direct *in vivo* imaging. Antisense strategies involve the use of radiolabelled oligonucleotides (RASONs). RASONs are small oligonucleotide sequences that are complimentary to a small segment of target mRNA or DNA, and could potentially target any specific mRNA or DNA sequence. RASON probes are being used to directly image endogenous gene expression at the transcriptional level. Aptomers are also small oligonucleotide sequences (RASONs) that can be used to target specific proteins. In this context, imaging specific mRNAs and proteins with RASONs provides a direct image of molecular-genetic events. Some efficacy for gamma camera and PET imaging endogenous gene expression using RASONs has been reported [7–10]. Nevertheless, RASON imaging has several serious limitations, including: (a) a low number of target mRNA/DNA molecules per cell; (b) limited tracer delivery (poor cell membrane and vascular permeability, can not penetrate blood-brain barrier); (b) poor stability (degradation by H-RNAse); (c) slow clearance (slow washout of non-bound oligonucleotides); (d) comparatively high background activity and low specificity of localisation (low target/background ratios). Imaging specific RASON targets in the body is complicated and interpretation of the images must be approached with caution.

Another example of a commonly used direct imaging paradigm is the visualisation of glucose utilisation using FDG (fluorodeoxyglucose) and PET [11,12]. FDG PET images largely reflect an enzymatic reaction; namely, the activity of hexokinase—the enzyme that converts glucose and FDG to glucose-6-phosphate and FDG-6-phosphate, respectively. Since phosphorylated FDG does not significantly cross the cell membrane and is relatively stable in the cell, the radiolabelled probe accumulates in the cell. FDG transport does influence the early PET images, but has only a small influence on

images obtained after 60 min. Sequential PET imaging and measurements of FDG accumulation in tissue (e.g. tumour) is essentially a radiotracer enzyme assay; and the FDG-PET imaging data can be used to provide information about hexokinase activity and be used to derive a measure of glucose utilisation [13–15]. This is an example of "direct" molecular imaging; it involves a radiolabelled probe (FDG) that is selectively metabolised (phosphorylated) and trapped in the cell by a single endogenous enzymatic reaction (hexokinase). Since mitochondrial hexokinase is a rate-determining step for glucose utilisation and the FDG-hexokinase enzymatic reaction determines the rate and magnitude FDG accumulation, PET FDG images can be used to measure glucose utilisation in the tissue.

2.2. Indirect imaging

Indirect imaging of endogenous gene expression is currently a widely used strategy. The paradigm was initially demonstrated using in situ optical imaging technology and reporter transgenes [1–3, 16]. In this paradigm, the reporter transgene (e.g. *E coli*, *β-galactosidase*) product is an enzyme (*β-galactosidase*) that cleaves a non-chromogenic galactoside (X-gal or β-gal) to a chromogenic precipitate that is localised to the site of reporter gene expression and appears blue on light microscopy [3,16]. This technology, however, requires post-mortem tissue sampling and processing for the β-galactosidase assay.

More recently, the principle of reporter gene imaging has been adapted to three non-invasive imaging technologies that have developed more or less in parallel; they include: (1) nuclear imaging (PET, single proton emission computed tomography (SPECT), gamma camera and autoradiography) [17–21], (2) magnetic resonance imaging [22–26], and (3) optical imaging (in small animals) [27–29]. Each imaging technology has specific advantages and limitations. For example, *in vivo* optical imaging is relatively inexpensive and can be performed repetitively in the same animal, but is largely limited to imaging small animals (e.g. mice or rats). Magnetic resonance has the advantage of very high spatial resolution, can be performed repetitively over time in the same animal or human subject, but is currently more limited with respect to the number and variety of molecular and reporter gene assessments. Nuclear imaging has the benefit of high sensitivity and localisation of radioactivity in 3-dimensional space; it can be used to directly image an assortment of biological processes as well as the expression of different reporter genes with highly specific radiolabelled probes; quantitative information can be obtained from the images and the imaging paradigms are directly transferable to large animals and human subjects. However, nuclear imaging technology is very expensive and multidisciplinary (e.g. requires a cyclotron and radiochemistry resources for PET imaging).

2.3. PET-based reporter gene imaging

Many PET-based molecular imaging paradigms are "indirect"; many currently use reporter-transgene technology and complimentary radiolabelled probes (specific complimentary radiolabelled probes are necessary to produce an image of reporter-transgene expression). Current PET-based reporter gene imaging paradigms fall into two categories: enzyme-based and receptor-based. The most widely used PET "reporter genes" are transcribed and translated to an enzyme; the enzyme selectively converts a complimentary "reporter probe" to a metabolite that is selectively trapped within transduced cells. The receptor-based reporter gene imaging paradigm involves a reporter gene product that is a receptor that "irreversibly" traps the complimentary probe in transduced cells.

Modern molecular techniques provide us with the ability to design specific reporter gene constructs of cDNA, where the reporter gene is placed under the control of upstream promoter/enhancer elements. These promoter/enhancer elements can be "always turned on" with constitutive promoters (such as long terminal repeat (LTR), respiratory syncytial virus (RSV), cytomegalovirus (CMV)), or they can be "sensitive" to activation by specific endogenous transcription factors (factors that bind to and activate specific enhancer elements). Since some enhancer elements are associated with specific endogenous genes, these enhancer sequences can be placed upstream of the reporter gene in a specifically designed reporter construct. The reporter construct can then be transduced into target tissue and used to monitor the activity and regulation of specific endogenous genes. An example of this imaging paradigm has recently been validated; it has been shown that transcriptional regulation of endogenous (host tissue) gene expression can be imaged with PET [5].

A general paradigm for reporter gene imaging using PET imaging technology is shown in Fig. 1. The herpes simplex virus type 1 thymidine kinase gene (*HSV1-tk*) with specific upstream promoter/enhancer elements is transfected into target cells by a vector. It is important to note that imaging transgene expression is independent of the vector used to transfect/transduce target tissue; namely, any of several currently available vectors can be used (e.g. retrovirus, adenovirus, adeno-associated virus, lentivirus, liposomes, etc.). Inside transfected cells, the *HSV1-tk* gene is transcribed to *HSV1-tk* mRNA and then translated on the ribosomes to a protein (enzyme), HSV1-TK. After administration of a complimentary radiolabelled reporter probe (5-iodo-2′-fluoro-2′deoxy-1-β-D-arabino-furanosyl-uracil (FIAU) or FHBG) and its transport into transduced cells, the

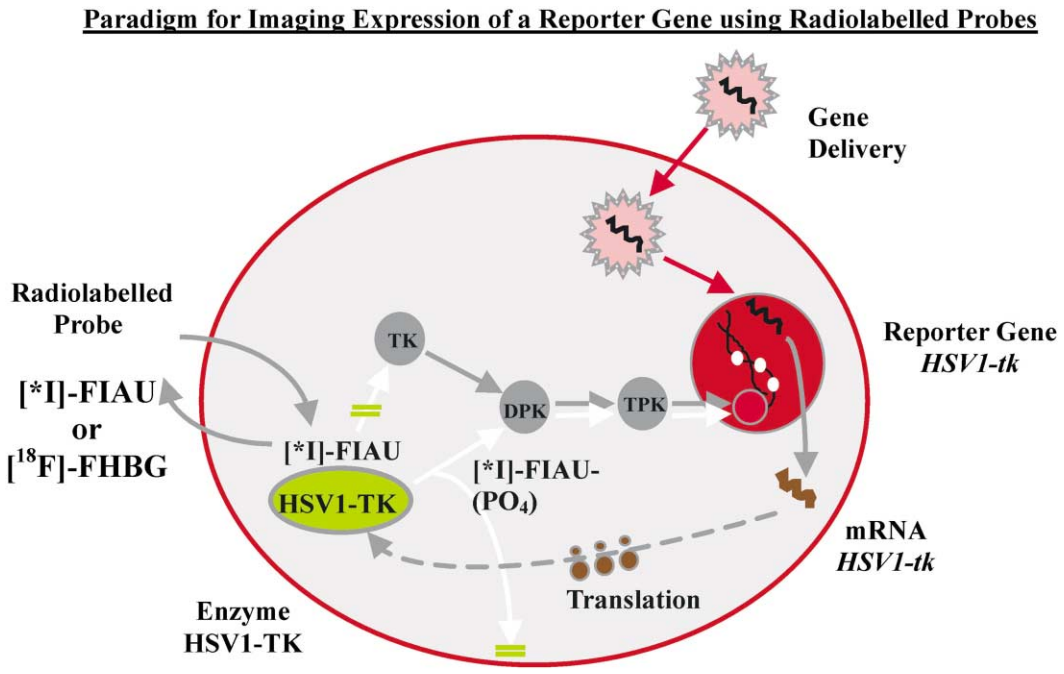

Fig. 1. Schematic for imaging herpes simplex virus type 1-thymidine kinase (*HSV1-tk*) reporter gene expression with reporter probes FIAU and FHBG. The *HSV1-tk* gene complex is transfected into target cells by a vector. Inside the transfected cell, the HSV1-tk gene is transcribed to *HSV1-tk* mRNA and then translated on the ribosomes to a protein (enzyme), HSV1-TK. After administration of a radiolabelled probe and its transport into the cell, the probe is phosphorylated by HSV1-TK (gene product). The phosphorylated radiolabelled probe does not readily cross the cell membrane and is ''trapped'' within the cell. Thus, the magnitude of probe accumulation in the cell (level of radioactivity) reflects the level of HSV1-TK enzyme activity and level of *HSV1-tk* gene expression.

probe is phosphorylated by HSV1-TK (gene product). The phosphorylated reporter probe does not readily cross the cell membrane and is ''trapped'' within the cell. Thus, the magnitude of reporter probe accumulation in transduced cells reflects the level of HSV1-TK enzyme activity and level of *HSV1-tk* gene expression. Enzymatic amplification of the signal (e.g. level of radioactivity) also facilitates imaging the location and magnitude of probe accumulation and reporter gene expression. It may be useful to consider this reporter-imaging paradigm as an *in vivo* enzymatic radiotracer assay that reflects reporter gene expression. Viewed from this perspective, reporter gene imaging is similar to imaging hexokinase activity with fluorodeoxyglucose (FDG).

Most reporter imaging studies using radiolabelled probes and PET involve wild-type *HSV1-tk* or mutant *HSV1-sr39tk* [30]. The *HSV1-tk* and *HSV1-sr39tk* gene products are proteins (enzymes) that have less substrate specificity than mammalian thymidine kinase 1 (TK1). They phosphorylates a wider range of compounds, including acycloguanosines (e.g. acyclovir, ACV; ganciclovir, GCV; 9-[4-fluoro-3-(hydoxymethyl)butyl]guanine, FHBG) and 2′-fluoro-nucleoside analogues of thymidine (e.g., 5-iodo-2′-fluoro-2′deoxy-1-β-D-arabino-furanosyl-uracil, FIAU). This difference in enzyme specificity permits the development and use of radiolabelled probes that are phosphorylated to a significantly greater extent by HSV1-TK or HSV1-sr39TK in comparison to mammalian TK1.

A reporter gene can also encode for an extracellular or intracellular receptor (e.g. hD2R, or hSSTR2) that ''irreversibly'' binds or transports a radiolabelled or paramagnetic probe. The human dopamine 2 receptor (hD2R) [31] and the human somatostatin receptor subtype-2 (hSSTR2) [32,33] genes have been suggested as potential reporter genes for human studies. Both human genes have limited expression in the body; hD2R expression is limited to the striatal-nigral system of the brain and high hSSTR2 expression is largely limited to carcinoid tumours. This approach is a very clever strategy because there are established complimentary radiolabelled probes for each of these reporter genes; 3-(2′-[^{18}F]fluoroethyl)spiperone (FESP) for hD2R imaging [34], and [^{111}In]DTPA-octreotide (a complimentary radiolabelled somatostatin analogue) for hSSTR2 imaging [35]. Furthermore, both probes are approved for human administration. Both of these reporter systems have distinct benefits with respect to initiating molecular/reporter imaging in human subjects. However, receptor expression on the surface of cells is a complex process and involves intracellular trafficking and cell membrane incorporation that is likely to be altered under different conditions and different disease states. It remains to be shown whether imaging receptor-based

reporter systems (e.g. the *hD2R* and *hSSTR2* reporter gene systems) will provide a consistent and reliable measure of reporter gene expression under variable stress or altered conditions. In any case, the level of probe accumulation (level of radioactivity) must be shown to be proportional to the level of gene expression.

3. Applications of PET-based reporter gene imaging

3.1. Monitoring gene therapy

A non-invasive, clinically applicable method for imaging the expression of successful gene transduction in target tissue or specific organs of the body would be of considerable value for monitoring and evaluating gene therapy in human subjects [36]. The reporter transgene(s) can be driven by any promoter/enhancer sequence of choice [37]. The promoter can be "constitutive" (leading to continuous transcription), or it can be inducible (leading to controlled expression). The promoter can also be cell-specific, allowing expression of the transgene to be restricted to certain cells and organs. In this way the reporter expression cassette can be designed to provide information about endogenous gene regulation, mRNA stabilisation and specific protein–protein interactions.

PET imaging could define the location, magnitude and persistence of gene expression over time. Targeting gene therapy to a particular tissue (e.g. tumour) or specific organs of the body is an increasingly active area of research. Several issues that are important for the clinical optimisation of gene therapy remain unresolved in many current clinical protocols: (1) Has gene transduction or transfection been successful?; (2) Is the distribution of the transduced or transfected gene localised to the target organ or to target tissue, and is the distribution in the target optimal?; (3) Is the level of transgene expression in the target organ or tissue sufficient to result in a therapeutic effect?; (4) Does the transduced or transfected gene localise to any organ or tissue at sufficient levels to induce unwanted toxicity?; (5) In the case of combined pro-drug- gene therapy protocols, when is transgene expression maximum (optimal) and when is the optimal time to initiate treatment with the pro-drug?; (6) How long does transgene expression persist in the target and other tissues?

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

3.2. Therapeutic gene imaging

HSV1-tk has the advantage of being both a "therapeutic gene" (combined with ganciclovir treatment) and a "reporter gene" (using an appropriate radiolabelled probe, such as FIAU or FHBG). This combination allows for direct imaging of the therapeutic gene product (HSV1 thymidine kinase), and can be used to define the location, magnitude and duration of *HSV1-tk* gene expression. Experimental validation of this approach has been demonstrated in animal models of colorectal metastases to the liver treated with adenoviral-mediated *HSV1-tk* gene transfer and ganciclovir ("suicide" gene therapy) [39,40], or treatment with conditionally replicating, oncolytic herpes viruses that constitutively express the *HSV1-tk* gene [41,42]. However, most therapeutic genes do not lend themselves to direct imaging of their transgene product. Furthermore, the development and validation of a new probe and a new imaging paradigm specific to each therapeutic transgene of interest would be a very costly and time-consuming endeavour. Many therapeutic gene products do not readily lend themselves to radionuclide assessments and it may not even be possible in some cases. It is therefore more reasonable to consider alternative strategies for "indirect" imaging of therapeutic gene expression that use established reporter gene–reporter probe combinations.

3.3. Reporter gene imaging

Several indirect reporter-gene imaging strategies have been described, and they can be used to image different therapeutic genes. These strategies take advantage of using established reporter gene–reporter probe combinations, and can achieve the objective of monitoring therapeutic gene expression without directly imaging/measuring the therapeutic gene product. One strategy uses a "fusion gene" containing cDNA from both the reporter and therapeutic genes [38]; a second strategy uses a "*cis*-linked" construct for proportional therapeutic and reporter gene expression [33,43,44]; a third strategy uses "multiple vectors", where one or more vectors carry a therapeutic gene and another vector carries the reporter gene [45].

The use of fusion-gene technology provides a single bicistronic transcription cassette for co-expression of two genes that are "linked" in a fixed definable manner

in a single, combined ''fusion gene''. One segment of the fusion gene cDNA corresponds to the ''therapeutic'' gene and another segment corresponds to the ''reporter'' gene. Transcription of the fusion gene occurs under the control of upstream promoter/enhancer regulatory elements of choice (e.g. constitutive expression or expression only in response to a specific activator that is present only in targeted organs or tumours). Translation of mRNA proceeds to yield a single ''fusion protein'' (the gene product) containing both reporter and therapeutic amino acid sequences. The fusion protein is a single hybrid of the two individual proteins. Thus, information obtained by imaging the ''reporter'' component will provide corresponding information about the ''therapeutic'' component [38]. However, there are a number of potential disadvantages of the fusion gene approach. The fusion construct may not result in a functional gene product. This could be due to a change in the conformational structure of the native protein or result in an alteration in the subcellular localisation of the fusion protein, or to a loss in activity of its ''therapeutic component'', or to a loss in activity of its ''reporter component''. Modulation of the fusion mRNA or a change in the clearance (breakdown) of the fusion protein may also be sufficiently different compared with the two native proteins (gene products of the two native genes). Such differences could have a significant impact on the level of the fusion gene product, and thereby, on the level of its biological activity. Fusion proteins are larger than each of the corresponding native proteins and are more likely to generate an immunological response. Thus, fusion gene technology cannot be generalised and may not be widely applicable in clinical imaging of therapeutic gene expression. However, when a fusion gene product is functional and non-immunogenic, it may be a very useful construct for monitoring therapeutic gene expression.

The use of *cis*-linked genes with an internal ribosomal entry site (IRES) element within a single bicistronic transcription cassette provides an alternative approach for achieving proportional co-expression of two genes. The IRES element enables translation initiation within the bicistronic mRNA, thus permitting gene co-expression by cap-dependent translation of the first cistron and cap-independent, IRES-mediated translation of the second cistron [33,43,44]. Successful imaging studies in animals using IRES constructs have been performed and indicate that proportional co-expression can be achieved [46,47]. However, cap-dependent translation of the first cistron has been shown to be several fold greater than cap-independent, IRES-mediated translation of the second cistern. Although gene product assays have shown proportional expression over a wide range in transduced cells and xenografts, it remains to be demonstrated whether IRES-based vector expression is a reliable indicator of transgene co-expression in different tissues. Namely, that the half-life of each encoded protein remains proportional in different tissues and organs of the body. This is necessary when non-invasive imaging is used to assess organ (tissue) specificity, as well as the level and duration of transgene expression.

The use of *multiple vectors* is another approach for achieving co-expression of one or more therapeutic genes and a reporter gene. In this paradigm, the genetic backbone of each vector (e.g. adenovirus) is identical, except for inclusion of a therapeutic gene or a reporter gene. Each vector would have identical envelope characteristics and identical promoter/enhancer elements driving the expression of the therapeutic and reporter transgenes. A multiple vector cocktail containing definable (different) combinations of the individual vectors can be administered. A key requirement for this approach is that: (1) the multiple vectors transfect and transduce target organs and tissue proportionate to their ratio in the administration cocktail; (2) the therapeutic and reporter genes are co-expressed proportionally in all target organs and tissue; and (3) the proportionality of co-expression is constant over a wide range of expression levels. A recent report has provided encouraging results in support of using multiple vectors (adenovirus) to deliver reporter and therapeutic genes to target organs [45].

3.4. PET-based reporter gene imaging of biological processes

Reporter-gene imaging is being used to visualise endogenous biological processes, including transcriptional and post-transcriptional regulation of target gene expression, as well as specific intracellular protein–protein interactions. Imaging transcriptional regulation of endogenous genes in living animals using non-invasive imaging techniques is providing a clearer understanding of normal and cancer-related biological processes. These new imaging paradigms can be directly translated into human studies using similar reporter constructs, radiolabelled reporter probes and PET imaging.

Several important biological processes are affected by endogenous expression of p53, including apoptosis. A recently published paper from our group [5] showed that p53-dependent gene expression can be imaged *in vivo* with PET. A retroviral vector (*Cis-p53/TKeGFP*) was generated by placing the herpes simplex virus type 1 thymidine kinase (*tk*)—enhanced green fluorescent protein (e*GFP*) fusion gene (*TKeGFP*, a dual-reporter gene) under control of a p53-specific response element. DNA damage-induced upregulation of p53 transcriptional activity was demonstrated in transduced cells and xenografts. The level of reporter expression (level of radioactivity in the transduced cells and xenografts) correlated with the expression of p53-dependent downstream genes (including *p21*). This was the first

demonstration that a *Cis*-reporter system (*Cis*-p53/TKGFP) was sufficiently sensitive to image endogenous gene expression using non-invasive nuclear (PET) imaging and [124I]FIAU (Fig. 2). The PET images corresponded with upregulation of genes in the p53 signal transduction pathway (p53-dependant downstream genes) in response to DNA damage induced by BCNU chemotherapy. PET imaging of p53 transcriptional activity in tumours using the *Cis*-p53TKGFP reporter system could be used to assess new drugs or novel therapeutic approaches, including gene therapy strategies based on p53 overexpression [48].

T-cell activation is an essential component of the immune response in normal and disease states. We recently demonstrated that it is possible to image T-cell receptor (TCR)-dependent activation of T-cells in vivo using PET [49]. A retroviral vector (*Cis*-NFAT/TKGFP) was generated by placing the fusion gene (*TKGFP*) under control of the response element for nuclear factor of activated lymphocytes (NFAT). A human T-cell leukaemia cell line (Jurkat) that expresses a functional T cell receptor (TCR) was transduced with the *Cis*-NFAT/TKGFP reporter vector and used in these studies. Known activators of T-cells (anti-CD3 and anti-CD28 antibody) produced significantly higher levels of *TKGFP* reporter gene expression (increased GFP fluorescence, increased levels of *HSV1-tk* mRNA and increased radiolabelled probe accumulation) in *Cis*-NFAT/TKGFP+ Jurkat cells compared with non-treated or non-transduced Jurkat cells. PET imaging of mice with local *Cis*-NFAT/TKGFP+ Jurkat cell infiltrates demonstrated significantly higher levels of radioactivity localised to the transduced and anti-CD3/CD28 stimulated Jurkat cell infiltrates compared with corresponding controls (Fig. 3). A strong correlation between *TKGFP* expression and upregulation of T-cell activation markers (CD69 and Interleukin-2 (IL-2) production) was demonstrated in both the *in vitro* and *in vivo* studies. These results demonstrated that: (1) activation of the NFAT signal transduction pathway occurs after TCR stimulation, and (2) PET imaging of T-lymphocyte activation in tumours following TCR engagement is feasible using the described *TKGFP*-based *Cis*-reporter system. This imaging paradigm could be used to assess the efficacy of novel anti-tumour vaccines and adoptive immunotherapy.

4. Issues for the future

Molecular imaging has its roots in both molecular biology and cell biology as well as in imaging technology (nuclear, magnetic resonance, optical, etc.). These disciplines have now converged to provide a well-established foundation for exciting new research opportunities and for translation into clinical applications. The development of versatile and sensitive assays that do *not* require tissue samples would be of considerable value for *in vivo* studies and the monitoring of molecular-genetic and cellular processes in animal models of human disease (including transgenic animals), as well as for studies in human subjects. Non-invasive imaging of

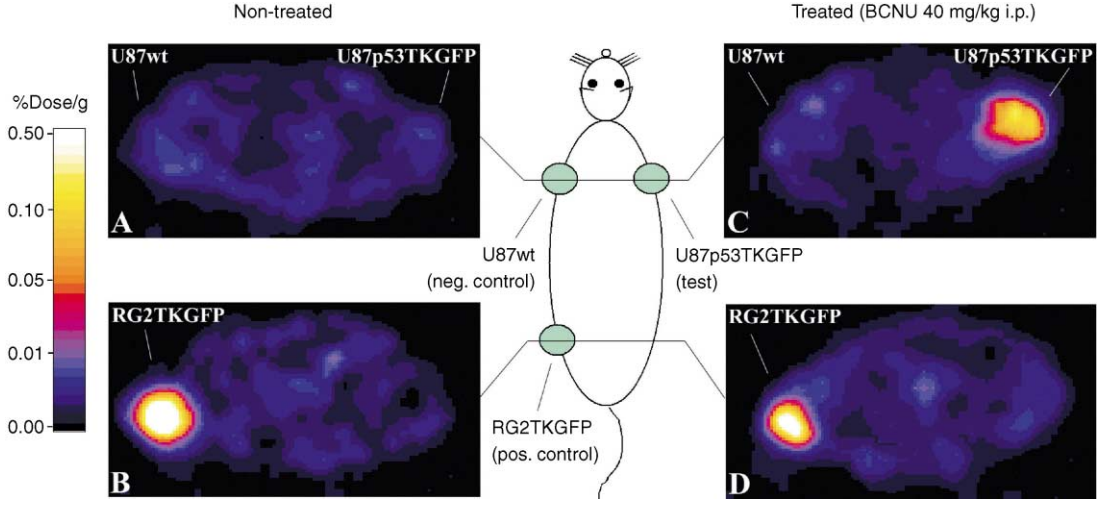

Fig. 2. PET imaging of endogenous p53 activation (from reference [5]). Transaxial PET images (GE Advance tomograph) through the shoulder (a, c) and pelvis (b, d) of two rats are shown; the images are colour-coded to the same radioactivity scale (%dose/g). An untreated animal is shown on the left, and a BCNU-treated animal is shown on the right. Both animals had three subcutaneous (s.c.) tumour xenografts: U87p53TKGFP (test) in the right shoulder, U87 wild-type (negative control) in the left shoulder, and RG2TKGFP (positive control) in the left thigh. The non-treated animal on the left shows localisation of radioactivity only in the positive control tumour (RG2TKGFP); the test (U87p53TKGFP) and negative control (U87wt) tumours are at background levels. The BCNU-treated animal on the right shows significant radioactivity localisation in the test tumour (right shoulder) and in the positive control (left thigh), but no radioactivity above background in the negative control (left shoulder). neg., negative; pos., positive; i.p.,intraperitoneally.

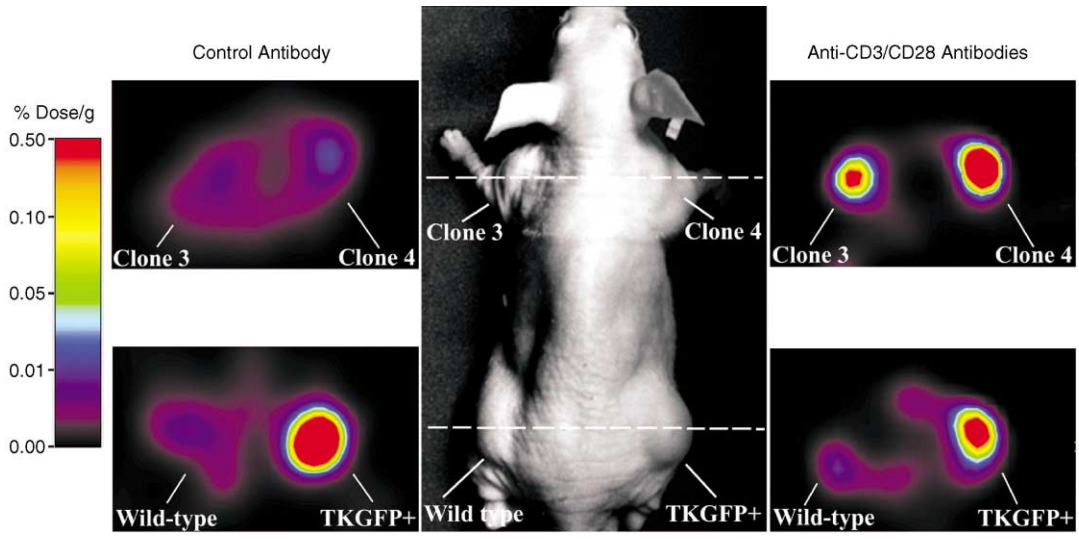

Fig. 3. Imaging NFAT-TKGFP reporter system activity with [^{124}I]FIAU and PET (from reference [49]). Photographic image of a typical mouse bearing different s.c. infiltrates (middle panel); transaxial PET images (GE Advance tomograph) of NFAT-driven TKGFP expression in a mouse treated with control antibody (left panel) and anti-CD3/CD28 antibodies (right panel) were obtained at the levels indicated by the dashed lines. [^{124}I]FIAU accumulation (%dose/g) is colour-coded to a range of values.

molecular-genetic and cellular processes will compliment established ex vivo molecular-biological assays that require tissue sampling, and would provide a spatial as well as a temporal dimension to our understanding of various diseases.

This new field of investigation has expanded rapidly over the past 5 years, particularly in the United States. This has been largely due to several National Cancer Institute (NCI)-sponsored initiatives. "Cancer imaging" was identified as one of six "extraordinary scientific opportunities" by the NCI in 1997–1998. Subsequent funding initiatives from the NCI have provided a major stimulus to further the development of *in vivo* molecular imaging. Substantial resources have been made available to the research community through NCI's Small Animal Imaging Resources Program (SAIRP) and the *In Vivo* Cellular and Molecular Imaging Centers (ICMIC) programme. Further interaction with other programmes, such as the Mouse Models of Human Cancers Consortium (MMHCC), is being encouraged and NCI is attempting to coordinate the yearly meetings of the SAIRP, ICMIC and MMHCC programmes. Similar funding initiatives have been developed by other NIH Institutes and by the Department of Energy (DOE). Furthermore, a new National Institute of Health (NIH) institute—the Institute for Biomedical Imaging and Engineering—has recently been formed to expand the breadth of the imaging community in the USA.

The opportunities for molecular imaging (and biomedical imaging as a whole) indeed look bright. Nuclear imaging with PET, SPECT, gamma camera and autoradiography will all contribute in their own way. However, it must be emphasised that nuclear imaging is very dependent on chemistry and radiochemistry, as well as on a reliable source of radionuclides. A multidisciplinary team is required for the development and synthesis of new radiopharmaceuticals and specific radiolabelled probes for molecular imaging. Further advances in nuclear-based molecular imaging research (and nuclear medicine in general) are highly dependent on exceptional radiochemistry. For example, many of the novel and exciting imaging paradigms are being explored with cost-effective *in vivo* optical imaging in mice. Translation to larger animals using PET and gamma camera imaging technology has already occurred and further extension to human studies is both feasible and imminent. This will require the development of appropriate reporter constructs (using relatively low cost, well established methods) and complimentary radiolabelled probes (requiring a high-cost multi-disciplinary team). Unfortunately, the cyclotron/radiochemistry infrastructure requirement is frequently not appreciated or ignored by administrators in many PET centres throughout the world. The cyclotron/radiochemistry infrastructure limitations must be corrected before nuclear-based imaging can fully develop at many PET centres.

Reporter gene imaging will be more limited in patients compared with that in animals due to the necessity of transducing target tissue with specific reporter constructs. Ideal vectors for targeting specific organs or tissue (tumours) in patients do not exist at this time, although vector development is a very active area of human gene therapy research. Each new vector requires extensive and time-consuming safety testing prior to government approval for human administration. Similarly, government approval is required for

human administration of new radiopharmaceuticals and radiolabelled probes. The translation of molecular imaging research into patient studies and clinical application must proceed step-wise and must be carefully monitored. The benefits of non-invasive monitoring (imaging) of transgene expression in gene therapy protocols are substantial and provide a stimulus to proceed with vigour. The ability to visualise transcriptional and post-transcriptional regulation of endogenous target gene expression, as well as specific intracellular protein-protein interactions in patients will provide the opportunity for new experimental venues for research in patients. For example, it may be possible to image the malignant phenotype of a patient's tumour at a molecular level (e.g. abnormal activity of transcriptional regulation of a specific gene, or activity of a particular signal transduction pathway). Similarly, it may be possible to image a drug's effect on a specific regulatory or signal transduction pathway in an individual patient's tumour. At the moment, this requires the use of reporter gene transduction vectors that target specific organs or tissue (tumours). Nevertheless, we remain optimistic; the tools and resources largely exist and we should be able to perform limited gene imaging studies in carefully selected patients in the near future.

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