Molecular Imaging of Gene Expression and Cell Trafficking

18

It is one of the more striking generalizations of biochemistry – which surprisingly is hardly ever mentioned in the biochemical text books – that the twenty amino acids and the four bases, are, with minor reservations, the same throughout Nature.

Francis Harry Compton Crick

18.1 Gene Therapy

The human genome consists of more than 3 billion base pairs and approximately 30,000 genes, most of which are conserved across the species (genetic universality). Natural interindividual variability (*singularity*), however, occurs in a substantial portion of the genome, giving rise to individual characteristics. Variability in the sequence of a particular gene locus (location) is known as a polymorphism and occurs when two or more forms (*alleles*) of the gene exist.

Gene therapy represents a new and promising therapeutic modality, which holds significant promise for the treatment of human diseases. Natural genes encode naturally occurring proteins and can be used to either complement an abnormal or deficient function, or to induce a new function in a target cell. In contrast, chimerical genes do not exist in nature but are encoded for engineered proteins designed to alter various biochemical functions of a cell. The main principle of gene therapy is based on achieving a controlled and effective target-specific expression of endogenous genes or *transgenes* (externally transferred genes into cells) in order to generate a beneficial biological effect, like a cure, or to slow down the progression of a disease, or even augment the growth of new cells (Miller 1992; Mulligan 1993). Several important genes involved in gene therapy protocols in oncology, cardiology, and neurology are listed in Table 18.1.

The goal of gene therapy sometimes is also to obtain genetically modified cells with a new or a restored function, and in other occasions genetic material is used to temporarily interfere with a cell function. The major goal, however, is to achieve a controlled and effective target-specific expression of genes. One of the important factors in the design of gene therapy strategies is the nature of the disease being treated. For certain diseases a specific organ may have abnormal pathology, while some other diseases may be systemic and involve several organs and tissues. Also, a disease may sometimes be caused by a single dominant gene, or a single recessive gene or multiple genes, as in the case of cancer.

Gene therapy protocols for cancer treatment are the most common approaches (Peñuelas et al. 2005a), however, this approach is also being used to treat other diseases in cardiology and neurology (Wu and la-Herttuala 2005; Inubushi and Tamaki 2007; Jacobs et al. 2005; Jeffrey et al. 2008).

One of the common approaches of gene therapy in cancer treatment is the suicide gene therapy, in which the genes (like non mammalian enzymatic genes) make the tumor cells more susceptible to a subsequently introduced *prodrug* that can be converted to a highly toxic active drug. The two most common suicide gene systems are Herpes Simplex Virus Type I *Thymidine Kinase* (HSV1-*tk*) (Moolten 1986) and *cytosine Deaminase* (CD) (Haberkorn et al. 1996). HSV1-tk is capable of phosphorylating pyrimidine and purine nucleoside derivatives which can be trapped intracellularly (Alrabiah and Sacks 1996). Following treatment, prodrugs, like acyclovir, gancyclovir, and pencyclovir, are phosphorylated and incorporated into the host DNA, in place of thymidine triphosphate. As a result, the cellular replication is blocked leading to a decrease in tumor growth. Similarly CD, a bacterial gene, converts 5-Fluorocytosine into the toxic chemical

S. Vallabhajosula, *Molecular Imaging: Radiopharmaceuticals for PET and SPECT,*
DOI: 10.1007/978-3-540-76735-0_18, © Springer-Verlag Berlin Heidelberg 2009

Table 18.1 Role of genes in different diseases

Clinical	Target condition	Mechanism	Genes
Oncology	Replacement of tumor suppression effect	Cell cycle regulation	p53
	Antisense strategy	Inhibit oncogene expression	
	Drug sensitization, prodrug therapy	Suicide genes	HSV1 *tk-1*
	Proapoptotic	Promote cell death	Caspases Bax, FasL,
	Angiogenesis	Factors inhibiting angiogenesis	Antisense VEGF and EGF
Cardiac disease	Ischaemic heart disease	Promoting angiogenesis	VEGF, FGF, HGF, HIF-1α
	Heart failure	Correction of calcium handling	SERCA-2a
		Restoration of β-adrenergic receptor signaling	β-adrenergic receptor, β-ARK1 inhibitor
		Modulation of apoptosis	Bcl-2, IGF-1, PI 3-kinase, *Akt*, p38α
	Post PTCA	Limitation of restenosis by endothelial repair	VEGF
		Limitation of restenosis through cell cycle regulation	NO synthase, VEGF, PDGF-P receptor
	Atrial fibrillation	Modification of atrioventricular nodal conduction	Inhibitory G protein α subunit
		Increase in automaticity	Kir 2.1, HCN2
Neurology	Cerebral Ischaemia and stroke	Modulation of apoptosis	Bcl-2, NAIP, GDNF
	Alzheimer's disease	Extending the survival of cholinergic neurons	NGF
		Prevention and reduction of Aβ burden	ApoE2
	Parkinson's disease	Preventing the degeneration of dopaminergic neurons	Alpha-synuclein (PARK1) and parkin (PARK2) genes
		Improving the production and release of striatal dopamine	TH, GCHI, AADC and VMAT-2
		Neuroprotection	GDNF, BDNF

SERCA Sarcoendoplasmic reticulum Ca^{2+}- ATPase; *VEGF* Vascular endothelial growth factor; *FGF* Fibroblast growth factors; *HGF* hepatocyte growth factor; *HIF* Hypoxia-inducible factor; *IGF* Insulin-like growth factor; *PI* Phosphatidylinositol; *PDGF* Platelet-derived growth factor; *NO* Nitric oxide; *PTCA* Percutaneous transluminal coronary angioplasty; *NAIP* Neuronal apoptosis inhibitory protein; *GDNF* Glial-derived neurotrophic factor; *BDNF* Brain-cell derived neurotrophic factor; *NGF* Nerve growth factor; *GCHI* GTP cyclohydroxylase I; *VMAT-2* Vesicular monoamine transporter type 2; *AADC* Aromatic amino decarboxylase; *TH* Tyrosine hydroxylase; *HCN* Hyperpolarization-activated cyclic-nucleotide-gated

5-Fluorouracil (5-FU), which substitutes for uracil in the cellular RNA, and prevents translation or protein synthesis (Habelkorn et al. 1996).

Although gene therapy is still in its infancy, monitoring the targeting and expression of genes is very important to the successful clinical applications (Serganova et al. 2007). The most challenging issues for applying gene therapy to human diseases are as follows (Sangro et al. 2002)

- The choice of a relevant therapeutic gene
- The appropriate selection of promoter and regulatory sequences driving the expression of the transgene
- The characteristics of the vector used for the delivery of the transgene into cells
- The development of systems for clinical monitoring of gene expression in the target organs.

18.1.1 Gene Delivery

The genetic material used for gene therapy has to be introduced into the target cells to exert the intended therapeutic effect. The term *transduction* refers to the incorporation of the foreign gene by the target cells and the expression of the corresponding gene product (Fig. 18.1). Genes do not penetrate inside the cells easily; they and have to be driven by gene therapy vectors. Ideally, these vectors should be able to harbor large

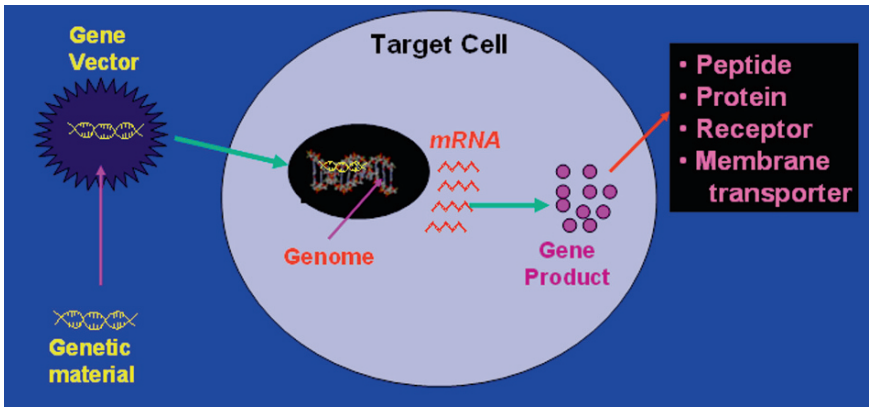

Fig. 18.1 The basic principle of gene therapy: Transduction of a therapeutic gene into a target cell and subsequent gene expression (gene product) leading to a therapeutic effect

DNA constructs with high transduction efficiency, allow for controlled and targeted gene expression, and be safe for both the patient and the environment. Many different vectors have been developed so far which can be divided into two broad categories: viral and nonviral vectors (De and Gambhir 2005).

Viruses are a natural delivery system for delivering their DNA or RNA. In viral vectors different portions of the wild-type viral genome have been replaced for the transgene of interest. Basically four different groups of viral vectors have been developed; Retrovirus (single stranded RNA), Adenovirus (double stranded DNA), Adeno associated virus (AAV), and Herpes Simplex Virus (HIV). The transgene capacity, however, varies from low (4.5 kb for AAV vectors) to medium (7–8 kb for retro- and lentiviral vectors) to high (36–150 kb for high-capacity adenovirus and HSV-1 amplicon vectors (Jacobs et al. 2005). Viral vectors are highly efficient for gene transfer, however, some are immunogenic while others have the inherent risk of inducing oncogenesis through integration in the host's genome. The nonviral vectors comprise a broad variety of vehicles for gene transfer including DNA–protein complexes, different formulation of liposomes (naked liposomes, immunoliposomes, virosomes), and naked DNA (direct injection or gene-gun mediated). The main advantages of these types of gene vectors are their larger DNA capacity, good toxic profile, and lack of immunogenicity (Peñuelas et al. 2004). In general, viral vectors, however, have substantial advantages over nonviral vectors, and therefore, are the most widely used vehicles for gene transfer in the clinical setting, to date.

Two general approaches have been used to deliver genes to the target cell: direct transfer of genetic material into cells in vivo and indirect methods involving the reimplantation of in vitro genetically modified cells (Peñuelas et al. 2004). The in vivo method of gene therapy is based on delivering the gene to a specific organ or tissue using a viral or nonviral *vector* that can be introduced into the patient by inhalation, intravenous, or even local administration. In the ex vivo method the cells from a tissue (to be treated) are removed from the patient, transfected with the therapeutic gene exvivo, and the transgenes are then reintroduced into the patient. Thus a diversity of natural (viruses) or artificial molecular constructs – named gene therapy vectors – are used to achieve efficient cell transduction.

18.1.2 Gene Expression

The process of gene expression of either endogenous or transfected genes involves the *transcription* of a gene into messenger RNA (*mRNA*), and subsequent *translation* of mRNA into a gene product or a protein (such as peptide, enzyme, receptor, membrane transporter) (Fig. 18.1). Transcription of a gene, however, is regulated by regulatory regions (such as promoters and enhancers), which are coded in the DNA. Universal promoters allow transgene expression in every transduced cell, while cell-type-specific regulatory proteins, known as *transcription factors,* interact with promoters and limit gene expression of specific cells.

18.2 Gene Imaging

In gene therapy imaging gene expression is important for monitoring the location(s), magnitude, and time-variation of gene expression from gene-therapy vectors, and also for measuring the efficacy of gene therapy. In vivo imaging techniques could, thus, play a pivotal role both, in preclinical and clinical research in gene therapy, since many crucial questions remain to be solved in this field. In an era of patient specific molecular medicine, the imaging technique should be able to provide quantitative information on many of the issues related to the gene therapy protocols (Peñuelas et al. 2004), such as:

- Has the vector used for gene transfer been efficiently delivered to the target organ/tissue?
- Is the distribution of vector in the target optimal?
- What is the biodistribution of intravenously administered vector?
- Did the gene transfer take place efficiently?
- Is the gene being expressed and the level of gene expression sufficient for the intended therapeutic effect?
- How long does gene expression persist both, in target and in nontarget tissues?
- What is the optimal moment for prodrug administration in "suicide" gene therapy protocols?
- Has the gene therapy protocol been efficient for the disease cure/remission?

The use of appropriate molecular imaging techniques, to trace the gene expression, is critical for determining the efficacy, or lack of efficacy of a given vector used for a specific gene therapy protocol. While optical imaging techniques are useful for evaluating the potential utility of gene therapy approaches in a wide variety of animal models, they are essentially not applicable in the clinic. Since the concentration of many of the gene products following transcription and translation are in the range of nano and picomolar levels, MRI techniques may not have the necessary sensitivity due to the mass limitations required for a useful MR signal. In contrast, because of their inherent high sensitivity of radiotracer imaging techniques, PET and SPECT may be more appropriate to identify the specific targeting of therapeutic genes, locate the magnitude of gene expression, and finally, monitor the response to gene therapy. Basically, two different strategies (Fig. 18.2) can be used to image gene expression (Gambhir et al. 1999a) as outlined below.

18.2.1 Direct and Indirect Gene Imaging

In direct gene imaging approach, the endogenous gene or transgene expression at the level of *m*RNA, or the subsequent gene products, (enzymes, receptors, etc) following translation, can be the specific target for developing radiolabeled probes (Fig. 18.2). Specific

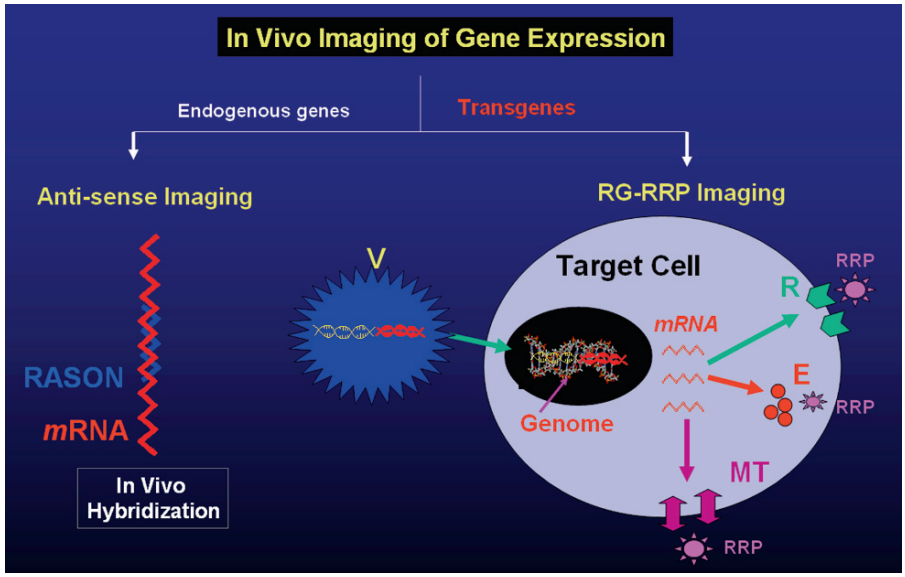

Fig. 18.2 In vivo imaging of gene expression of two types (**a**) in vivo hybridization of endogenous gene expression based on RASON, (**b**) imaging transgene expression based on a radiolabeled reporter gene probe. The gene product can be a receptor (R), an enzyme (E), or a membrane transporter (MT)

sequence of *m*RNA can be targeted for imaging using a radiolabeled antisense oligonucleotide (RASON), probe, such as ^{18}F labeled oligonucleotide, containing a complimentary sequence of mRNA to be imaged. Similarly, to the target a specific gene product or therapeutic protein, radiolabeled substrates for that protein can be used. While this approach has potential advantages, it may not necessarily be practical since each and every mRNA or gene product would require a specific radiolabeled molecule.

The indirect gene imaging approach involves coupling the therapeutic gene (TG) to a "reporter gene (RG)", such as HSV1-*tk*, and then targeting the reporter gene expression using a PET or SPECT "reporter probe (RP)" (Tjuvajev et al. 1996, Gambhir et al. 1999b). The TG and the RG are linked with a common promoter and administered into a patient using a virus (such as adenovirus) vector, which transfers both the therapy gene and RG to the target tumor cell. Subsequently, a radiolabeled RP (RRP) is used to image the expression of RG which indirectly will provide information about the expression of TG (Fig. 18.3). This RG-RRP approach may be used based on a gene for the production of a specific enzyme or an intracellular and/or cell-surface receptor in the target cell.

18.2.1.1 Antisense Imaging

mRNA molecules are typically several hundreds to thousands of base pairs long and the levels of mRNA in the cytoplasm are in the range of 1–1,000 pM. Antisense oligonucleotides (ASON) have a nucleotide base sequence complementary to a specific sequence of a small segment on the target mRNA molecule (Fig. 18.4). The interaction of *m*RNA and ASON is very specific and stoichiometric resulting in the *hybrid*ization through hydrogen bonds. In order to image intracellular levels of mRNA, small ASON molecules (12–35 bases) have been modified to develop radiolabeled antisense oligonucleotide (RASON) probes (Hnatowich 2000; Tavitian 2000). The validity of antisense imaging using RASON probe was first demonstrated based on an ^{111}In-DTPA-oligonucleotide targeted against *c-myc* oncogene (Dewanjee et al. 1994). Subsequently several RASON probes, based on ^{99m}Tc,

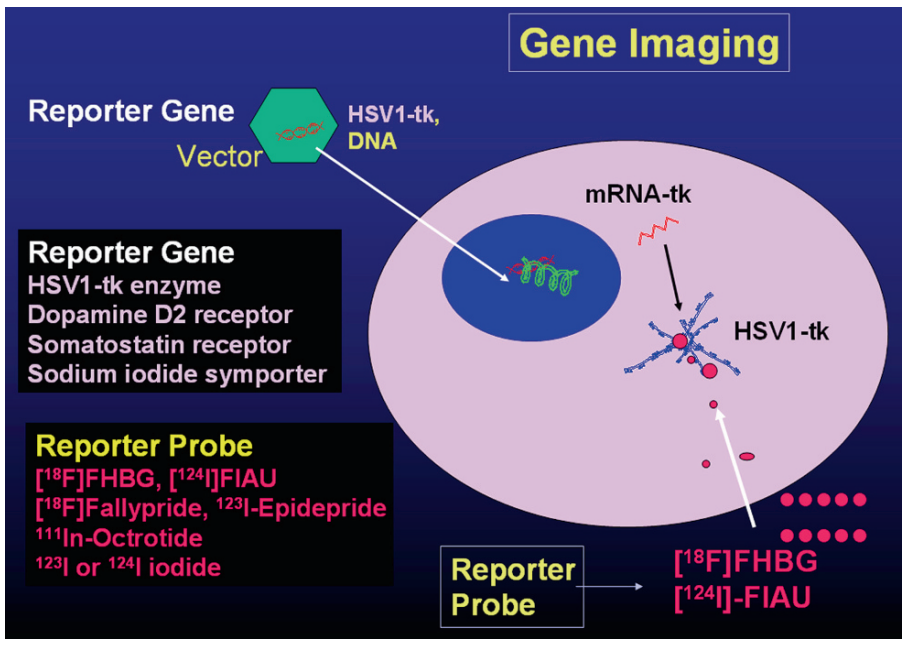

Fig. 18.3 Imaging transgene expression first involves transduction of a vector consisting of both the therapeutic gene and the reporter gene (such as HSV1-tk) into a target cell. Subsequent imaging of the gene product HSV1-*tk* enzyme, using reporter probe, such as [^{18}F]FHBG or ^{124}I–FIAU

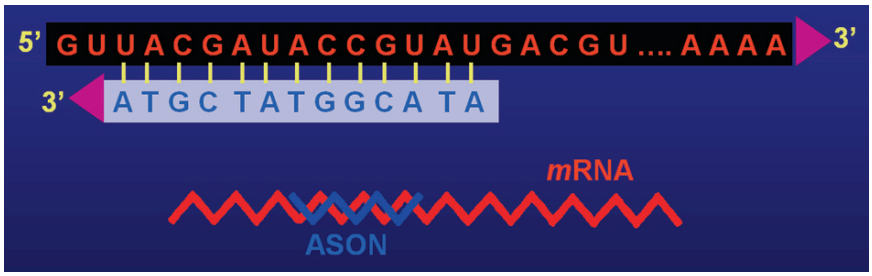

Fig. 18.4 Antisense oligonucleotides (ASON) have a nucleotide base sequence complementary to a specific sequence of a small segment on the target mRNA molecule. The interaction of *m*RNA and ASON is very specific and stoichiometric resulting in the *hybrid*ization through hydrogen bonds

^{125}I and ^{18}F nuclides were evaluated in animal models to assess the utility of antisense imaging technique to monitor endogenous gene expression. The major requirements for a RASON probe are as follows (Barrio 2004):

- The complementary base pairing in the RASON probe must be very high and specific. Even a single mismatch can decrease the hybridization significantly.
- The SA of the RASON probe must be very high (1–10 Ci μmol^{-1}).
- Since oligonucleotides are charged molecules, membrane permeability may be a limiting factor. Membrane transport process must be optimal and favorable for the kinetics of cellular uptake and washout.
- Intracellular nonspecific binding must be relatively low or insignificant.
- Peripheral metabolism must be minimal.

18.2.1.2 Reporter Probe Imaging

Several human and non-human RGs have been proposed (Table 18.2) as potential candidates for developing gene imaging technique based on radiolabeled reporter probes (RRP). The RGs can be classified into three categories, based on whether a gene product is an enzyme, a receptor, or a membrane transporter. The HSV1-*tk* viral gene has been the most widely exploited RG in preclinical studies. Since this is a nonhuman gene, there is a small risk of generating an immune response against cells and tissues transduced with this gene. Therefore human genes may be more advantageous compared to the viral genes. Also several human genes have been identified which can generate significant amounts of specific membrane receptors (such as SSTR, dopamine D2) and transporters (NIS, NET). A number of PET and SPECT radiotracers have already been developed for various receptors and transporters, and tested in humans as molecular imaging probes. Potential signaling and the cell biology effects of using receptors or transporters as RGs, however, may limit their potential clinical utility.

Table 18.2 Imaging transgene expression in vivo: Human and nonhuman reporter genes and the corresponding radiolabeled reporter probes

Class	Reporter gene	Reporter probe
Enzymes	*TK2, HSV1-tk*,	[^{124}I]FIAU, [^{18}F]FEAU, [^{18}F]FHBG, [^{18}F]FHPG
	HSV1-sr39tk	[^{18}F]FEAU
Receptor	Sodium iodide symporter (NIS)	[^{124}I]Iodide, [^{123}I]Iodide
	Norepinephrine transporter (NET)	[^{123}I]MIBG, [^{124}I]MIBG
Receptor	Somatostatin receptor (SSTR2)	^{68}Ga-DOTATOC, ^{111}In-DTPA_Octrotide
	Dopamine D2	[^{18}F]Fallypride, ^{123}I-epidepride

In order to develop the gene imaging technique, it is important to establish a method that would successfully generate expression of both the TG and the RG. Both of these genes can be given separately in separate vectors or can be linked together and administered in a single vector. It is also important to choose an appropriate promoter (P) driving the transcription. The following approaches have been developed to administer the TG and RG (De and Gambhir 2005) (Fig. 18.5).

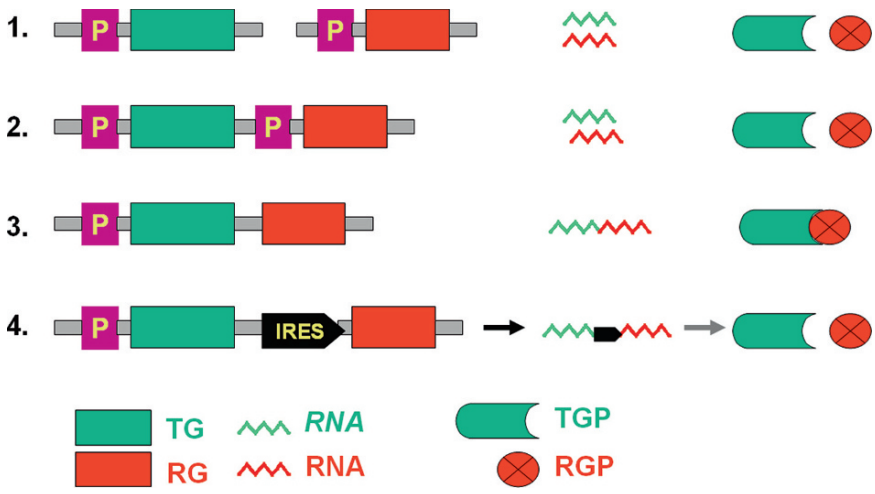

Fig. 18.5 Different approaches to administer therapeutic gene (TG) and reporter gene (RG). (1) Coadministration approach, (2) Fusion gene approach, (3) Dual promoter approach, and (4) Bicistronic approach. The gene expression involves both the therapeutic gene product (TGP) and the reporter gene product (RGP)

- Coadministration approach: the two genes are carried in two identical but distinct vectors. As a result two mRNAs and two distinct proteins are generated.
- Fusion gene approach: The two gene sequences are coupled together with a spacer sequence between them. As a result only one mRNA leading to one fusion protein is generated with both therapeutic and reporter functions.
- Dual promoter approach: The two genes are linked but two identical promoters are used, one for each gene. As a result two mRNAs with two distinct proteins are generated.
- Bicistronic approach: The two genes are linked together using an internal ribosomal entry site (IRES), a specific sequence to initiate translation. Transcription occurs under a single promoter to form a single mRNA molecule. However, translation under the direction of IRES generates two separate proteins.

Each of the approaches discussed above has its own advantages and disadvantages. Selection of an appropriate approach may depend on a particular combination of TG and RG. The ultimate goal is to image and quantitatively estimate the levels of TG expression.

The interaction of radiolabeled reporter probes (RRPs) with a specific gene product may lead to a significant trapping of RRPs within the cell or in a specific tissue or organ of interest. The ideal characteristics of any RG-RRP imaging approach should have the following minimum characteristics (Gambhir 2004).

- The RG should preferably be a human gene, and generally not be expressed in the target tissue of interest.
- The levels of RG expression (gene product) must be relatively high in the target tissue to allow significant trapping of RRP.
- The SA of RRP must be high (1–10 Ci μmol^{-1}) in order to detect even minimal levels of gene products (the specific targets).
- RRP should be stable in vivo, and rapidly cleared from blood and nonspecific binding sites.
- The radionuclide used for developing the RRP must be appropriate (a) to determine the kinetics of trapping of RRP in the target tissue and (b) to quantitatively estimate the levels of gene expression.

Enzyme-Based Reporter Genes

The expression of several RGs coding for different enzymes including β-galactosidase, luciferase, or green fluorescent protein (GFP) have been extensively studied in transgenic animals. However, measuring the expression of these genes requires a biopsy or the killing of the animals. The bioluminescence optical approaches with a firefly or luciferase provide rapid throughput in small animal models, but are not applicable for human studies. In contrast, HSV1-tk gene expression has been shown to be useful for developing noninvasive imaging strategies.

With HSV1-tk, the final gene product generated is an enzyme (protein), known as HSV1-tk. In contrast to the human intracellular thymidine kinase 1 (*hTK-1*), the HSV1-tk enzyme is less specific to thymidine, and has the ability to phosphorylate other nucleoside analogs (acyclovir, ganciclovir, and penciclovir) to their corresponding monophosphate metabolites (Keller et al. 1981). Since human *TK-1* can not phosphorylate these nucleoside analogs, the viral *TK* enzyme permits the development and use of radiolabeled probes that are selectively phosphorylated by the HSV1-*tk* enzyme. A mutant HSV1-*tk* RG was also developed which generates the HSV1-sr39*tk* enzyme that also has shown specificity for nucleoside analogs (Gambhir et al. 2000b).

Two main substrate groups have been used to develop RRPs for HSV1-*tk*. Among the pyrimidine analogs, the best radioiodinated (^{123}I, ^{124}I or ^{131}I) substrate with high affinity for the HSV1-*tk* enzyme is 5-iodo-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-iodouracil (FIAU) (Tjuvajev et al. 1998). FIAU however, is also a good substrate for *hTK-1*. The best substrate among the acycloguanosine derivatives is 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl) guanine (FHBG) (Alauddin and Conti 1998). Compared to FIAU, FHBG is a poor substrate for the endogenous *hTK-1*. FHBG has shown to be an even better substrate for the mutant HSV1-sr39tk enzyme (Gambhir 2004). The structures of several substrates for the human and viral TK enzyme are shown in Fig. 18.6.

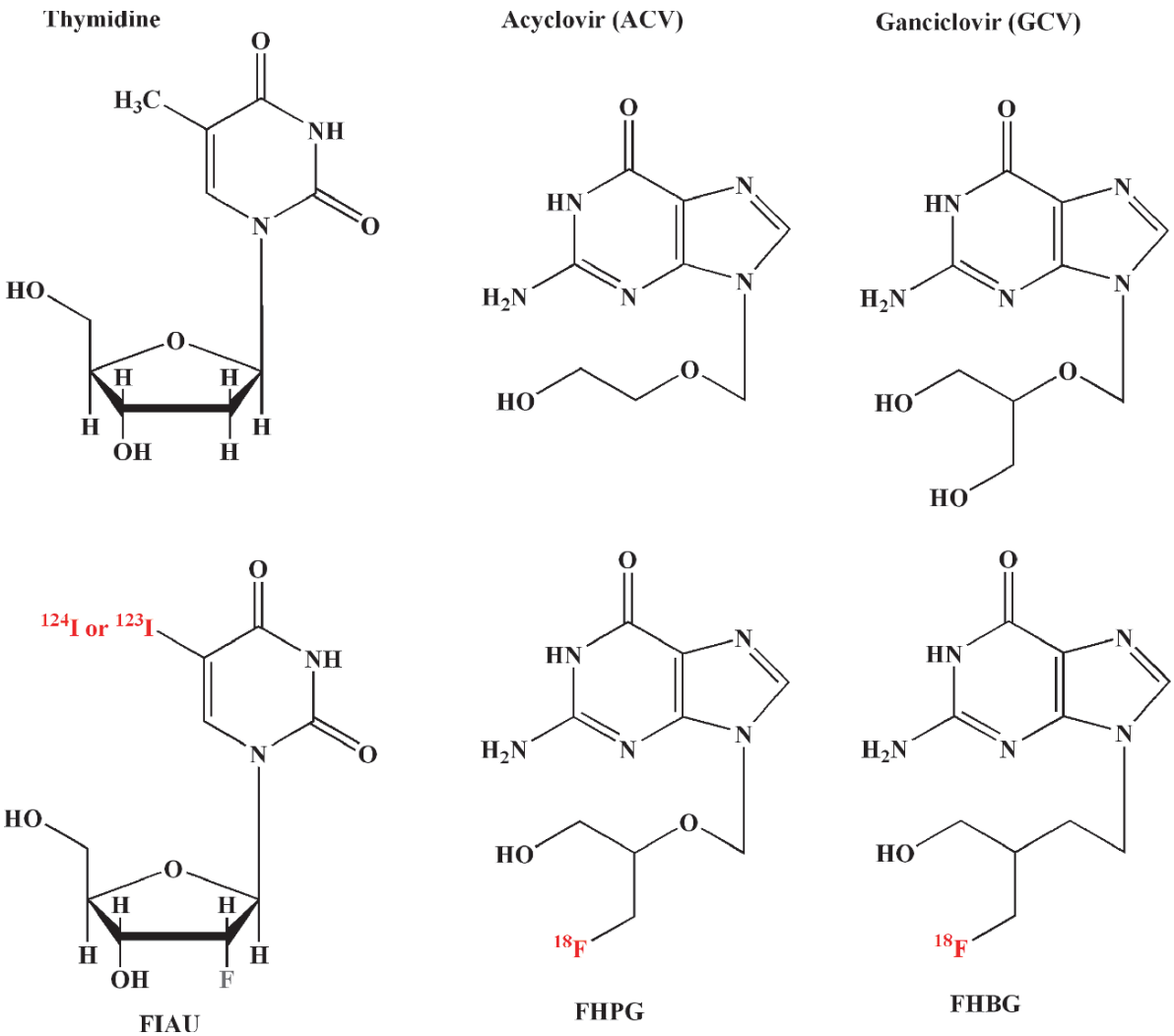

Fig. 18.6 Structure of several radiolabeled reporter probes for imaging the HSV1-tk enzyme

Since viral TK genes are not ideal for human studies, the human mitochondrial thymidine kinase 2 (*hTK2*) gene has been proposed as an appropriate RG (Ponomarev et al. 2007). There are several biochemical features of *hTK2* that make it an attractive RG. A truncated version of *hTK2* (Δ*hTK2*), which lacks the 18 N-terminal amino acids that are responsible for the mitochondrial localization of this protein, has been recently shown to function as a better reporter gene than the native *hTK2*.

Receptor-Based Reporter Genes

Specific membrane receptors have limited expression in the body and provide several advantages for gene imaging studies. Since the genes for the receptors are of human origin they are not immunogenic. Further, because the receptors are present on the cell membrane, the RRP does not require transport into the cell. Two receptor-based RG systems – somatostatin receptors and dopamine receptors – have been described and evaluated in preclinical studies (Zinn and Chaudhuri 2002; Serganoa et al. 2007).

The human somatostatin receptors subtype 2 (SSTR2) are mainly present in the pituitary gland and overexpressed in several neuroendocrine tumors. The human *SSTr2* gene has been incorporated into an adenoviral vector and has been used as a RG for noninvasive imaging of xenografts (Rogers et al. 1999, 2005). A number of SPECT and PET radiotracers for the SSTR2 system have already been developed and tested in clinical studies. Among these, ^{111}In-DTPA-octreotide (OctreoScan®), ^{99m}Tc-P829 peptide, and ^{68}Ga-DOTATOC can be used as RRPs since they are known to have very high SSTR2 subtype specificity (Serganoa et al. 2007).

The human dopamine D_2 receptors (D_2R) are predominantly present in the nigrostriatal system of the brain. The D_2R gene has been used as RG both, in the adenoviral delivery vector and in stably transfected tumor cell xenografts. The receptor expression has been imaged using 3-(2′-[^{18}F]fluoroethyl)-spiperone (FESP), a D_2R antagonist (MacLaren et al. 1999). In addition, using a rat dopamine D_2R and its mutant variant as an RG, the gene expression has been imaged using FESP. The nonhuman origin of mutant D2R does not favor their use in human studies. Therefore, there is a need for further work in developing mutant genes for human receptors in order to use receptor-based RGs.

Membrane Transporter-Based Reporter Genes

In contrast to receptors that usually have a 1:1 binding relationship with an RRP, membrane transporters provide signal amplification (similar to enzymes) through the transport-mediated concentrative intracellular accumulation of RRPs. Two human transporter genes, that are potentially good candidates for RG applications, have been reported: the human sodium iodide symporter (*hNIS*) gene and the human norepinephrine transporter (*hNET*) gene (Barton et al. 2003; Anton et al. 2004; Serganoa et al. 2007).

The *NIS* is an intrinsic transmembrane glycoprotein that is mainly expressed in the thyroid, however, lower levels of expression are also present in the salivary glands, stomach, thymus, breast, and other tissues (Dai et al. 1996; Dadachova and Carrasco 2004). NIS will transport a number of different anions, including iodide (I^-) and pertechnetate (TcO_4^-). The potential for using hNIS as a RG in human studies is well recognized (Mandell et al. 1999; Boland et al. 2000; Niu et al. 2004). Further, the hNIS gene has been successfully transferred into cultured cells and small animal tumors, and the symporter gene products have successfully accumulated both [^{131}I]iodide and ^{99m}Tc pertechnetate (Haberkorn et al. 2001; Che et al. 2005).

hNET is a transmembrane protein that mediates the transport of norepinephrine, dopamine, and epinephrine across the cell membrane (Pacholczyk et al. 1991). It functions as a rapid reuptake membrane transporter located at or near presynaptic terminals and facilitates the rapid reuptake of norepinephrine that was released into the synapse by the presynaptic terminals. hNET is a homo/oligomer complex, where Na^+ and Cl^- ions provide an inward electrochemical gradient and ATP/ATPase are critical elements for neurotransmitter accumulation. Successful imaging of hNET expression in neuroendocrine tumors and altered sympathetic enervation of the heart based on norepinephrine analogs (^{131}I–MIBG, [^{11}C]HED) have led to the suggested application of hNET as a human RG (Altman et al. 2003; Anton et al. 2004; Buursma et al. 2005; Moroz et al. 2007). Consequently, new and better norepinephrine analogs for imaging studies are under development (Ding and Fowler 2005).

18.2.2 Gene Imaging: Clinical Studies

Gene therapy has emerged as a new and promising therapy for many diseases (Haberkorn 2002; Isner 2002; Brewster et al. 2006; Lowenstein et al. 2004), and hundreds of clinical trials have been designed for the treatment of inherited disorders and a wide variety of acquired diseases. The majority of these clinical trials are, in fact, devoted to the treatment of cancer (Edelstein et al. 2004). Molecular imaging techniques for monitoring gene expression have been developed and successfully used in many animal models (Jacobs et al. 2005; Peñuelas et al. 2005a, b; Wu and la-Herttuala 2005). While the initial pilot studies in human subjects have demonstrated the safety and potential use of gene imaging technique based on RRPs, the sensitivity, reproducibility, and potential clinical utility, however, need to be tested and validated by carefully designed multicenter studies (Peñuelas et al. 2005a, b; Serganova et al. 2007). The pilot clinical studies of gene imaging technique based on RRPs for several different diseases are briefly discussed here.

The first clinical use of [^{124}I]FIAU–PET in a gene therapy procedure in humans may be considered as a

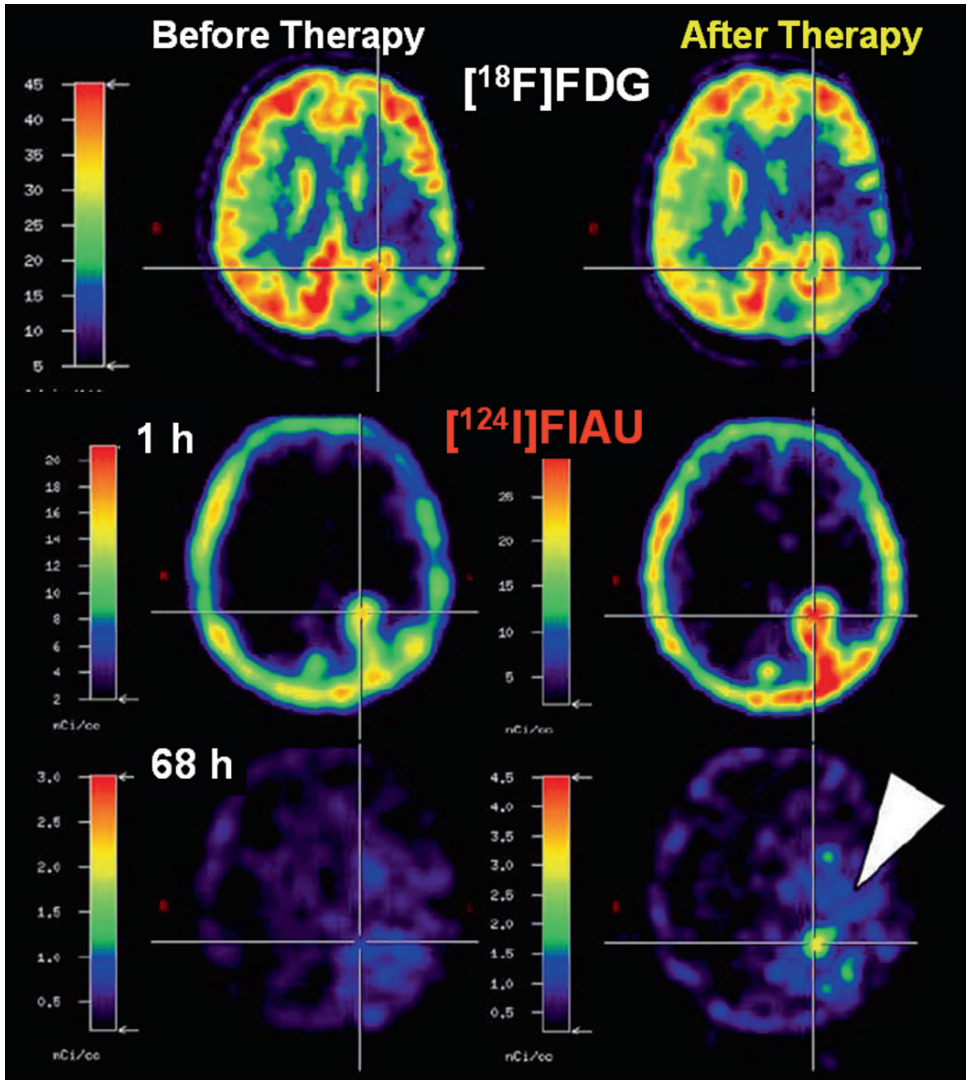

Fig. 18.7 PET imaging studies with FDG and ^{124}I–FIAU to monitor gene therapy (intratumorally infused liposome-plasmid gene therapy vector followed by ganciclovir administration) in a patient with a brain tumor. FDG–PET scan post therapy shows a central area of decreased uptake (due to necrosis) compared to the scan before therapy. Following administration of the ^{124}I–FIAU, the delayed image (at 68 h) shows a tumor retention of ^{124}I activity, indicating successful gene expression (Jacobs et al 2005)

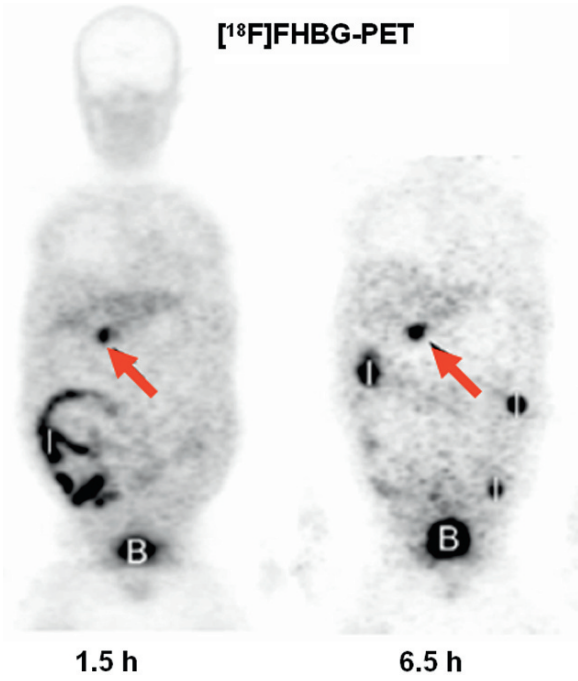

Fig. 18.8 [^{18}F]FHBG PET whole-body images show that specific accumulation of the tracer (and hence HSV1-tk expression) is restricted to the vector (10^{12} viral particles) injection site in the liver tumoral nodule (arrows). Later (6.5 h) the tracer accumulates in the bladder (B) and the intestines (I) owing to physiological elimination (Peñuelas et al 2005b)

typical example of the use of molecular imaging in gene therapy (Jacobs et al. 2001, 2005). More specifically, in patients with glioma, a phase I study was designed to evaluate the safety and potential therapeutic action of intratumorally infused liposome–plasmid DNA complex followed by the administration of ganciclovir (GCV) using intratumoral catheters. The initial identification of biologically active target tumor lesion was based on MRI and PET. A dynamic ^{124}I–FIAU–PET series acquired over three days was performed before the gene transduction and after the gene therapy to monitor the success of the gene delivery. The images in Fig. 18.7 show an increase in the accumulation rate of ^{124}I–FIAU in a single patient after intratumoral infusion of a vector encoding HSV1-tk. In addition, a FDG–PET study after gene therapy showed a significant decrease in FDG tumor uptake and central necrosis in the same area where ^{124}I–FIAU–PET confirmed gene delivery and expression.

In a larger series of patients, HSV-1-tk expression in tumoral hepatocarcinoma nodules treated by suicide gene therapy, was demonstrated using [^{18}F]FHBG, a tracer specific for HSV-1-tk (Peñuelas et al. 2005b).

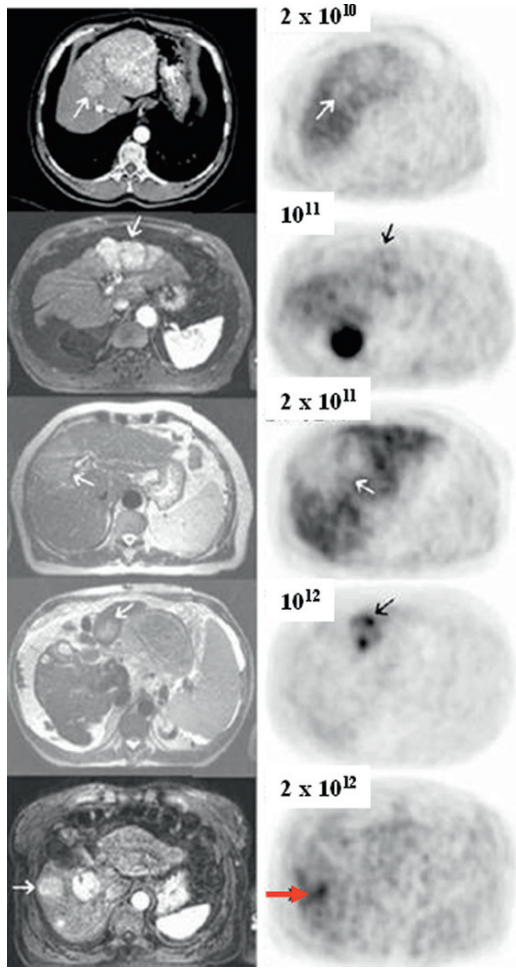

Fig. 18.9 Viral dose-dependent imaging of HSV1-tk transgene expression with [^{18}F]FHBG–PET in five patients receiving five different doses of viral particles (2×10^{10}–2×10^{12}). Detectable tumor transduction can only be observed when the dose of vector administered reaches a certain threshold of 10^{12} particles (Peñuelas et al 2005b)

Patients received the vector (1.0×10^{10}–2.0×10^{12} particles) by direct injection into the tumor nodule two days prior to the FHBG–PET; some patients had several FHBG–PET scans following multiple doses of therapy gene vector. Based on these preliminary studies, the following three findings were reported: (a) the signal to background ratio between the lesion and the nontreated liver increases with time after injection of the FHBG tracer (Fig. 18.8), (b) the magnitude of radiotracer accumulation in the treated lesion varies from patient to patient and cannot be directly correlated with the adenoviral dose used in each particular case (Fig. 18.9), and (c) the second dose of gene vector did not result in FHBG tumor localization, probably

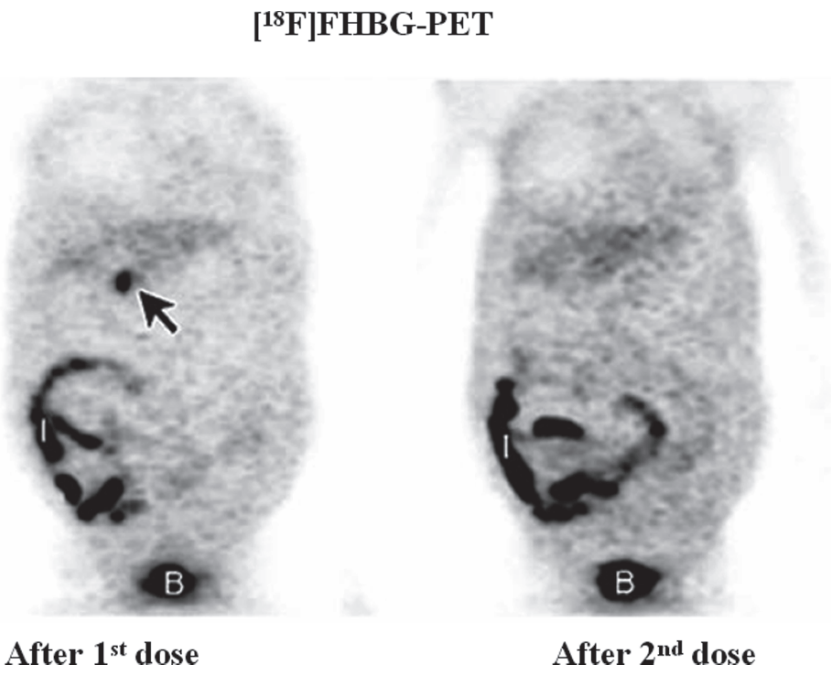

Fig. 18.10 [^{18}F]FHBG–PET whole-body images of the same patient two days after the first dose of the vector and two days after a second dose given 30 days after the first one are shown. No transgene expression can be observed after the retreatment in the tumor nodule, although it is evident in the first treatment (*arrow*). Accumulation in the intestines (I) and bladder (B) can be seen in both cases due to the physiological elimination of the tracer (Peñuelas et al 2005b)

due to increase in the titer of neutralizing antiadenovirus antibodies (Fig. 18.10).

In summary the preliminary clinical studies discussed above demonstrate that transgene expression, in cancer patients, can be monitored by PET. This noninvasive methodology represents a valuable tool for the assessment of gene expression in gene therapy in the clinical setting, and could be useful to (a) define the transduction efficiency of a given vector (in a specific tissue or lesion), (b) study transgene expression distribution, to determine its duration, and (c) help in the evaluation of new vectors and the design of novel therapeutic strategies.

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