Imaging in Gene Therapy at Molecular Level : A Review

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Abstract

Non-invasive Imaging in Gene Therapy has emerged as an essential tool for identifying the fate of vectors in tissues, quantifying injected dose in various organs, evaluating toxicity, and analyzing therapeutic gene expression level and treatment outcome. In the field of gene therapy, a considerable effort is being invested in the development of noninvasive imaging of gene expression. Several methods are available for non-invasive imaging of gene delivery and transgene expression, including magnetic resonance imaging (MRI), single photon emission tomography (SPECT)/positron emission tomography (PET), and fluorescence and bioluminescence imaging. Modern imaging techniques provide an opportunity to monitor direct vascular gene therapy.

This article presents the various strategies currently being developed, the methodologies in use and highlights recent key approaches using the latest imaging modalities in gene therapy. its clinical applications in the field of research with its future trends.

Key words: Non-invasive Imaging, Gene Therapy, Gene markers, Vectors

Introduction

Recently, much interest and speculation has been directed towards the use of non-invasive medicine imaging techniques to monitor gene expression. Many of the ideas expressed represent a shift in the strategy for nuclear imaging, with the suggested target being the actual messenger ribonucleic acid (mRNA) of specific genes, instead of the more classical approach of targeting the final products of gene expression, receptor, structural or enzymatic proteins, for imaging[1].

Non-invasive in vivo imaging has emerged as an essential tool for identifying the fate of vectors in tissues, quantifying injected dose in various organs, evaluating toxicity, and analyzing therapeutic gene expression level and treatment outcome. For example, non-invasive imaging of cancers involving head and read region, central nervous system, tumor, and cardiovascular tissues could verify gene expression in actual target tissues, resulting in better therapeutic approaches[1].

Some methods, such as computed tomography and magnetic resonance imaging (MRI), rely on energytissue interactions, whereas single photon emission tomography (SPECT) and positron emission tomography (PET) require the administration of reporter probes. However, in both computed tomography and MRI, exogenous contrast agents can be exploited to enhance contrast or follow biological markers[1].

Current imaging methods in gene therapy can be divided into two classes: transduction and biodistribution imaging. Transduction imaging visualizes transgene-mediated protein production; biodistribution imaging visualizes the actual systemic distribution of gene delivery vectors.[2,3]

Imaging Cellular Biology

Most of the current oncologic imaging technologies rely on macroscopic physical, physiologic or metabolic changes that differentiate tumors from normal tissue rather than identifying DNA mutations or specific DNA sequences. However, as genes and their functions are further defined in vitro, progress is being made toward imaging these events . Several modalities, including SPECT, PET, MRI, and optic imaging, indicate that it is feasible to image gene expression . In recent preclinical studies, several research centers have noninvasively imaged the delivery of exogenous genes and the expression of exogenous protein.[4]

Background On Gene Therapy

The goal of gene therapy is to supplement or replace the function of mutated genes with the correct genetic code. Rather than altering the disease phenotype by using agents that interact with gene products, or are themselves gene products, gene therapy can theoretically modify specific genes to correct the underlying cause of the disease. Gene therapy initially was envisioned for the treatment of inherited genetic disorders but is currently being studied in a wide range of diseases, including cancer, peripheral vascular disease, arthritis, and neurodegenerative disorders.[2]

Various gene transfer systems have been developed to penetrate the cell and deliver DNA to the nucleus where a therapeutic or marker protein can be expressed. These systems include replicant deficient viral vectors (such as adenovirus, adeno-associated virus, retrovirus, and herpes simplex virus; synthetic nonviral vectors (such as liposomes, polylysine, dendrimers, and molecular conjugates); physical methods such as the gene gun and electroporation naked DNA and combinations of these various technologies.[2]

Both ex vivo and in vivo protocols have been developed to deliver genes. For ex vivo gene therapy, the patient's cells are extracted and the gene is inserted into the cells in vitro and then are readministered to the patient. For in vivo delivery, the gene is transferred directly to the site of interest[2].

Many gene therapy protocols to date have concentrated on treatments for cancer. Though many cancers have a genetic predisposition orthey all involve acquired mutations. In general, cancers have at least one mutation to a protooncogene (yielding an oncogene) and mutations of at least one to a tumor suppressor gene, allowing the cancer to proliferate. The range of different cancers encountered and the mutations they

carry have led to a variety of strategies for gene therapy. namely genetic immunization, oncogene inactivation, tumor suppressor gene replacement, molecular chemotherapy, and drug resistance genes.[4]

Methods To Detect Gene Expression In Vivo in Preclinical Models

Two types of methodologies that are already used in medicine for other purposes are currently being adapted to usage in gene therapy: nuclear medical methods and magnetic resonance tomography. More recently, a technology exploiting the fact that bioluminescence can travel through tissues and be detected by very sensitive cameras has been developed.[3]

Three types of reporter genes are currently being considered and developed: enzymes, receptors, and transport proteins.

Enzymes

The reporter gene can be an enzyme expressed inside the cell that alters a labelled compound. The first tracers were developed for cytosine deaminase but cellular uptake proved slow and this system was superseded by an evolution of tracers that have been created for the prodrug-activating enzyme herpes simplex virus-thymidine kinase (HSV-1-Tk. Radiolabelled derivatives have been produced that can be used for imaging. Currently, FPCV (8-[18F] fluoro-penciclovir) is the tracer compound that enables the highest sensitivity even with weak expression of HSV-1-*tk*. A mutated HSV- 1-*tk* (HSV1-sr39*tk*) featuring a higher specificity to acycloguanosines such as FPCV provides a further increase in sensitivity[12].

Receptor Binding

Much experience exists in the imaging community with tracers that bind to surface receptors. These receptors can be expressed as transgenes and can serve as reporter genes together with their specific labelled ligand.[7]

Dopamine receptor

The dopamine D2 receptor can be expressed as a transgene in the cell membrane to induce binding of the ligand FESP (3-(2_-[18F]fluoroethyl)spiperone) which can be imaged by positron emission tomography (PET).

Somatostatin receptor type 2 (SSTR2)

Radiolabelled somatostatin analogues, such as [111In]octreotide, are routinely used in the clinic for the detection of rare neuroendocrine tumours expressing the SSTR2.

Transporter Proteins: The Na/I symporter (NIS)- Transport proteins have high specificity for certain compounds and can be expressed in the cell membrane as reporter genes. They use active transport to concentrate the labelled compound in a defined compartment such as the cell cytosol.

What And How To Image

To image specific molecules in vivo, several key criteria must generally be met (Fig 1)[4]

(*a*) availability of high affinity probes with reasonable pharmacodynamics;

(*b*) the ability of these probes to overcome biologic delivery barriers (vascular, interstitial, cell membrane);

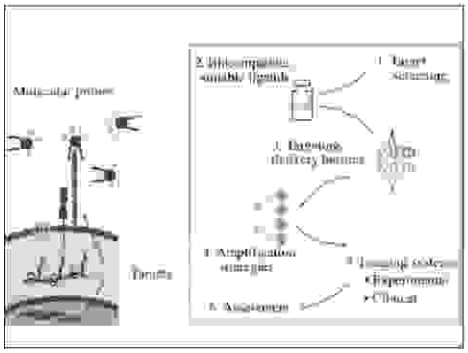
(*c*) use of amplification strategies (chemical or biologic).

(*d*) availability of sensitive, fast, highresolution imaging techniques.

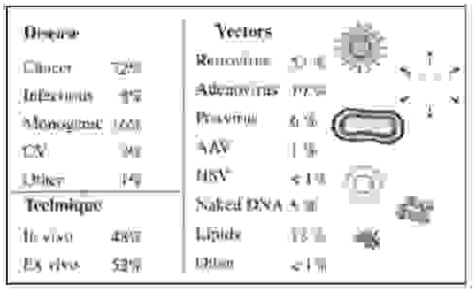
In a typical scenario, all four prerequisites must be met for successful in vivo imaging at the molecular level

Imaging Marker Genes

Marker Protein*	Imaging Modality	Ligand or Substrate*	Mechanism
Intracellular			
Thymidine kinase (11, 42-45	Nuclear	FIAU, ganciclovir, penciclovir, FHPG, FMAU	Phosphorylation of prodrug
Cytosine deaminase (48)	Nuclear, MR spectroscopy	Cytosine, fluorinated prodrugs	Deamination of prodrug, fluorine imaging
Tyrosinase (49)	Nuclear, MR imaging	Tyrosine, dopa	Oxidation of substrates into melanin or metal
Arginine kinase (5)	MR spectroscopy	ATP and arginine	Conversion to phosphoarginine
Creatinine kinase (51)	MR spectroscopy	ATP and creatinine	Conversion to phosphocreatinine
β-Galactosidase (14)	MR imaging	Galactosylated chelators	Cleavage of galactose residues changes relaxivity
Green fluorescent protein (52)	Optical	None required	Fluorescence
Luciferase (53)	Optical	Luciferin	Bioluminescence
Proteases such as cathepsin D (54)	Optical	Quenched near-infrared-fluorescent fluorochromes	Fluorescence activation
Cell surface			
Gastrin-releasing-peptide receptor (51, 140)	Nuclear	Bombesin	Affinity binding
Somatostatin receptor (57)	Nuclear	Repudes	Affinity binding
Dopamine-2 receptor (58)	Nuclear	^{18} spiperone	Affinity binding
Iodine binding (59)	Nuclear	Iodine	Trapping
Fusion proteins (60)	Nuclear	^{99m}Tc chelates	Transchelation
Engineered Internalizing receptor (61)	MR imaging	Transferrin	Internalization, relaxivity



(Fig 1) Schematics show prerequisites to in vivo molecular imaging. Potential targets can be at the DNA, RNA, or protein level.[4]



Schematic shows clinical gene therapy trials broken down by disease, technique, and vector system used. *AAV* 5 adeno-associated virus, *CV* 5 cardiovascular, *HSV* 5 herpes simplex virus.[4]

Use of Current Imaging Technology For Molecular Imaging

Non invasive imaging methods currently offer the greatest potential to be translated into clinical applications. They are highly sensitive, provide good time resolution, and acceptable spatial resolution. But alternative methods are currently in development[2].

MRI Imaging In Gene Therapy

MRI offers the advantages of high spatial resolution and the ability to measure more than one physiological parameter using different pulse sequences[3]. The underlying principle of MRI is nuclear magnetic resonance: unpaired nuclear spins (usually hydrogen atoms from water or organic compounds) align themselves when they are placed in a magnetic field, forming a net effect called net magnetization. In gene therapy applications, volumetric MRI can be used to measure the volume of a tumor. This approach provides high-resolution images with versatile contrasts and clear delineation of tumor borders[1].

MRI contrast agents

Contrast agents make it possible to detect gene delivery vectors and provide anatomical and morphological information, as well as information about the quantity of viral vectors or cells. [4a] Contrast agents are chemical substances introduced into the anatomical or functional regions to be imaged to increase the contrast between intact and abnormal tissues by altering relaxation times.

Contrast agents used in MRI can be divided into two categories: mainly T1-affecting agents with a positive contrast (increasing the signal in T1-weighted MRI) and mainly T2-affecting agents with a negative contrast (decreasing the signal in T2-weighted MRI).

Typical T1 contrast agents are low-molecular weight compounds containing a lanthanide, such as gadolinium & T2 contrast agents, different iron oxide nanoparticles have proven efficiency in biomedical imaging[4].

The MR contrasts generated by super-paramagnetic iron oxide (SPIO) particles have a diameter exceeding 50 nm and ultra-small paramagnetic iron oxide (USPIO) have a diameter of less than 50 nm particles are usually negative. Positive contrast has the clear advantage in gene therapy applications that the underlying anatomical features are better visualized

Paramagnetic chelates that change their magnetic properties at enzymatic hydrolysis ("smart" contrast agents) have also been used for gene expression imaging.[7,8] In one approach, the enzymatic activity of β-galactosidase was utilized to remove a blocking group of galacto-pyranose and alter the contrast properties of the probe.

Micro-MRI

Micro-MRI can be used in a wide variety of applications, including anatomical, functional, and molecular imaging.[4,9]

- Micro-MRI is often used to image the brain because of its ability to non-invasively penetrate the skull.
- Because of its high resolution, micro-MRI can also detect early small-sized tumors.
- Antibody-bound paramagnetic nano particles can also be used to increase resolution and to visualize molecular expression in the system

Pet Imaging In Gene Therapy

Because of its high sensitivity, PET is widely used for receptor imaging, particularly for the central nervous system[5,6]. PET can also be used to monitor the efficacy of gene therapy vectors. Reporter gene imaging can provide quantitative transduction data for viral vectors, spatial data about the transduction pattern and persistence of the vector used, and even data on endogenous molecular events in various reporter gene systems[10].

Among the many reporter genes suitable for PET imaging , the thymidine kinase transgene from HSV (HSV-*tk*)[11] and its mutant version HSV-*sr39tk*37 have been used extensively. HSV-*tk*, which is also used in anti-cancer treatments to promote apoptosis, has been successfully imaged with different radionuclides, including iodinated or fluorinated acycloguanosines (*e.g.*, ganciclovir, penciclovir, 18F-labeled 9-[4-fluoro-(hydr oxymethyl) butyl] guanine)[12] and thymidine analogues (*e.g.*, 2′- fluoro-2′-deoxy-1-beta-d-arabinofuranosyl-5-iodouracil and 2′-[18F] fluoro-5-ethyl-1-beta-d-arabinofuranosyl-uracil). These imaging tracers freely cross the plasma membrane by means of an active transport mechanism.

With viral imaging, lentivirus transduction *in vivo* has been followed using 1-(2-fluoro-2-deoxy-beta-d-arabinofuranosyl)- 76Br- 5-bromouracil (76Br-FBAU) as the imaging agent. In liver cancer patients, PET imaging showed that adenovirus-mediated HSV-*tk* expression disappeared within 9 days in all patients, aiding the assessment of clinical efficacy

The human dopamine receptor (human *D2R*) acts as cell membrane transporter or reporter gene. Human *D2R* expression is largely limited to the striatal-nigral system of the brain, which makes it a good candidate for transduction imaging with

norepinephrine transporterbased reporter system has been used to image transduced tumors[11].

Another marker gene, human sodium iodine symporter (human *NIS*, an iodine transporter in thyroid follicular cells), allows both PET (124I-iodine) and SPECT (123I-iodine or 99mTc) imaging. Human *NIS* has been used widely in gene therapy.

Micro-PET

- Micro-PET is usually widely used in clinical oncology & almost any biological compound can be traced.[1,27]
- 18F-FDG is used
- Micro-PET is also extremely sensitive to molecular details, and thus only nanograms of molecular probes are needed for imaging.

Spect Imaging In Gene Therapy

Although it is less sensitive than PET, SPECT offers a wider temporal range of imaging; it allows viral biodistribution imaging for up to several days using viral particles labeled with radioactive compounds.[5]

As with PET, various marker genes can be used with SPECT imaging. For example, HSV1-*tk* expression can be visualized using a suitable radioprobe, such as 123I-FIAU[14]. Similarly, lentiviral HSV-*tk* expression in fetal monkeys has been imaged using 18F-BHBG[15].

Although SPECT offers some advantages over PET and MRI, the resolution of isotope imaging limits the mapping of anatomical coordinates at the organ level.[6] In addition, several radiotracers accumulate not only in the target but also in excretion organs such as the liver, intestine, bladder, and stomach.

Typically, clearance times are 12 hours, after which the background signal from both blood and these organs is diminished. To produce more accurate anatomical data for nuclear imaging, multimodality imaging combining X-ray computer tomography with PET/SPECT imaging has largely replaced single-mode imaging in clinical use,[26] and MR-PET imaging is being developed[16].

Micro-SPECT

- Micro-SPECT is often used in cancer research for molecular imaging of cancer-specific ligands & brain because of its penetration power.
- Newer radioisotopes as 99mTC-labelled iron oxide nanoparticles.Isotopes are readily available,cheaper & images several molecular events simultaneously.[6,23]

Optical Imaging In Gene Therapy

Although MRI and SPECT/PET offer a wide range of imaging methods, the administration of radioactive ligands may be problematic in *in vitro* and *in vivo* imaging.[1] In addition, the use of the same marker gene both *in vitro* and *in vivo* offers the benefits of simpler study design and lower-cost imaging set-up[1].

Optical imaging can be divided into two categories:[1]

- **Fluorescence Imaging**
- **Luminometric Imaging**

In **fluorescence imaging** the external light source excitates a fluorochrome and the energy is emitted at a different wavelength when the fluorochrome returns to its ground state. **Green fluorescent protein (GFP)** from the jellyfish **Aequorea victoria** and its color variants[18] are among the most widely used marker genes in biomedical research.

In **luminometric imaging** the energy is obtained from a chemical reaction and light is released enzymatically at a specific wavelength. In both imaging modalities, the emitted light is usually detected using a charge-coupled device detector that can detect very weak signals. bioluminescence imaging enzymes are invisible to detectors until they catalyze the formation of products. **Luciferase enzymes** can catalyze the production of photons in the visible range of the spectrum in an oxygen/ substratedependent manner with a short half-life in cells.

However, the current applications of optical imaging are still limited to small animals and to the vicinity of surfaces in larger animals and humans. There are some fundamental drawbacks to optical imaging: the efficiency of light transmission through tissues is limited, quantification is not simple, and the signal may be hindered by light absorption by hemoglobin, limiting the depth of imaging.

Bioluminescence imaging suffers from limitations in terms of tissue penetration similar to those of fluorescence imaging, and thus has the problem of reduced clinical applicability. Whereas visible light penetration in tissues is only 12 mm, near-infrared light with wavelengths of 7001,200 nm enables better penetration into tissues, especially with the shorter wavelengths[24].

Ultrasound Imaging In Gene Therapy

Ultrasound images are obtained from the reflection of high frequency sound waves from surfaces between different tissues. Ultrasound devices are widely available and inexpensive, and they have a very short temporal resolution.Contrast-enhanced ultrasound uses gas-filled microbubbles to increase the backscatter for perfusion and blood flow analysis[7].

In gene therapy, contrast-enhanced ultrasound can be used for physiological imaging (e.g., imaging the restoration of perfusion after therapeutic vascular gene transfer)[10,20]. Organ-specific transfection has also been reported with targeted microbubbles. Recently, retroviruses have been enclosed in microbubbles and used for targeted gene delivery.

With ultrasound, up to 40-μm resolution within 5 mm of the detector can be achieved[21]. Limitations of the method include air and bone artifacts and limited depth of penetration at the higher frequencies required for imaging small organs in rodents.

Micro-ULTRASOUND

- Micro-ultrasound is the only real-time imaging modality per se, capturing data at up to 1000 frames per second.[25]
- Extremely cost-effective.
- Currently, imaging of up to 30 μm is possible,allowing the visualization of tiny vasculature in cancer angiogenesis microbubbles can be conjugated to markers such as $\alpha_v\beta_3$ integrin and vascular endothelial growth factor receptors (VEGFR), in order to provide molecular visualization

Application Of Non Invasive Imagining In Gene Therapy Concerned With Research Priorities[1]

While there are some aspects of development that are highly specific for a single mode of imaging (e.g.,MRI, PET, SPECT, OPTICAL & ULTRASOUND), there many areas of common ground for which there are general recommendations:

- Imaging researchers and basic scientists should work in close proximity to achieve the primary goal of relevant clinical studies.
- Developing imaging probes to study the functional interaction with contemporary molecular targets should be a priority.
- Imaging should be used to study the biodistribution of new drugs, which can be facilitated by labeling with radioisotopes, stable isotopes, or chromophores.
- Animal studies, including the use of gene knockouts as models for target-probe interactions, are useful tools for translational research, particularly with the use of small animal imaging.
- The time course of the imaging signal is usually very complex, and further development of mathematical algorithms and modeling is necessary to extract the maximum amount of quantitative pharmacologic information from each imaging procedure
- More emphasis is needed on the design of probes with respect to their ligand specificity and metabolic stability in order to minimize nonspecific signals during imaging.

Future Trends In Gene Therapy Imaging

In the future it will be important both to improve accurate signal localization and to increase sensitivity with high temporal resolution by using several imaging modalities with the same coordinates. Advances in materials will provide new imaging methods, as seen with the creative use of nanotechnology to image gene transcription[22], high-performance nanoparticles for ultrasensitive imaging[23], and highly sensitive hyperpolarized biosensors for MRI[24]. In addition, enhanced traditional materials, such as quantum dots[25] and far-red fluorochromes, could improve the specificity and efficiency of biodistribution imaging[26].

Together with new methods and materials, better imaging modalities will be available to a larger number of researchers, and the technology is being developed to increase anatomical accuracy and signal quantification. Efforts to combine different modalities, such as MRI and PET, will help to achieve these goals. It is likely that advanced imaging techniques will greatly enhance the development of more efficient and safer gene transfer methods for clinical use.

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