Non-invasive genetic imaging for molecular and cell therapies of cancer

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Abstract Gene therapy is a very attractive strategy in experimental cancer therapy. Ideally, the approach aims to deliver therapeutic genes selectively to cancer cells. However, progress in the improvement of gene therapy formulations has been hampered by difficulties in measuring transgene delivery and in quantifying transgene expression in vivo. In clinical trials, endpoints rely almost exclusively on the analysis of biopsies, which provide limited information. Non-invasive monitoring of gene delivery and expression is a very attractive approach as it can be repeated over time in the same patient to provide spatiotemporal information on gene expression on a whole body scale. Thus, imaging methods can uniquely provide researchers and clinicians the ability to directly and serially assess morphological, functional and metabolic changes consequent to molecular and cellular based therapies. This review highlights the various methods currently being developed in preclinical models.

Key words Imaging • PET • SPECT • MRI • Bioluminescence


Introduction

The invasive nature of classic pathological techniques precludes the possibility of a repetitive monitoring in the same subject. The results obtained from multiple biopsies from one patient or from animals sacrificed at different time points may be flawed by individual variations and they require a large number of samples to achieve statistical significance of the results. This is in addition to another shortcoming of invasive methods, that is, they cannot be applied in a clinical setting, especially in biopsy-restricted organs (i.e., brain and heart), making molecular imaging an attractive field of research.

Molecular imaging offers many unique opportunities to study biological processes in intact organisms. Many different technologies have been developed such as X-ray diffraction, electron microscopy, autoradiography, optical imaging, positron emission tomography (PET), magnetic resonance imaging (MRI), X-ray computed tomography (CT), single photon emission computed tomography (SPECT) and bioluminescence imaging (BLI), in order to image the structure and function of several systems, and to study gene expression in living subjects, with promising applications, as well as limitations to each. This review focuses on some of these molecular imaging technologies: PET, SPECT, BLI and MRI, and their roles in integrative imaging on mammalian biology from mouse to human; normal biological processes and their failure in disease are targets of these imaging methods.

PET

PET is a novel imaging tool, which uses compounds labelled with positron-emitting radioisotopes as molecular probes, to image and measure biochemical processes of mammalian biology in vivo. Some radioisotopes, for ex-
ample: oxygen ($^{14}\text{O}$, $^{15}\text{O}$), nitrogen ($^{13}\text{N}$) and carbon ($^{13}\text{C}$), can be administered to a subject and detected externally, as they are all positron emitters, as are Cu, Zn, K, Br, Rb, I, P, Fe and Ga, which are also used. There is no positron emitter of hydrogen, so fluorine-18 is used as a hydrogen substitute.

Initially described in 1995, PET was first used to administer a radiolabelled probe that is selectively bound or metabolised (e.g., phosphorylated) and trapped by interaction with the gene product, frequently an enzyme, in the reporter gene-transduced cell [1]. In this manner, the bound or metabolised probe accumulates selectively in the transduced tissue, and the level of probe accumulation is proportional to the level of gene product being expressed. Alternatively, the reporter transgene can encode for an enzyme (e.g., HSV1- tk), a receptor (e.g., the human dopamine 2 receptor, hD2R, and the human somatostatin receptor subtype 2, hSSSTR2) or a transporter (e.g., the human sodium iodide symporter, hNIS), that irreversibly traps the probe in transduced cells.

PET permits non-invasive visualisation of molecular and biological events in a living subject. The process of interest may be the expression of a specific gene, determination of the fate of putative stem cells after transplantation [1], upregulation of a specific cell protein [2–4], concentration of receptors on the cell surface [5, 6], decrease in regional perfusion [7], increase in glucose utilisation, decrease in oxygen consumption [8] or a whole host of other possible events. The goal of a PET assay is to accurately quantitate one or more of the processes through the use of novel positron-labelled probes (tracers) as well as appropriate image acquisition, data analysis and data modelling methodology.

In a typical molecular assay, a positron-labelled probe is injected intravenously, and PET scans provide measures of the probe tissue concentration and labelled products over time. These data are combined with a time course measurement of the plasmatic probe concentration, representing its delivery to tissue, and processed with a compartmental model containing equations describing the transport and reaction that the probe undergoes. The result is an image of the rate process under study.

Over 500 molecular imaging probes have already been developed and consist of various labelled enzymes, hormones, antibodies, peptides, drugs and oligonucleotides, but just two reporter gene approaches are discussed here: the Herpes simplex virus type 1 thymidine kinase gene (HSV1- tk) and the dopamine type 2 receptor (D2R).

First reporter gene: herpes viral thymidine kinase

HSV1- tk is a good example of a reporter gene because it is essentially non-toxic in humans and it has been used for cancer gene therapy approaches for decades.

The viral TK has a relaxed substrate specificity compared to the mammalian TKs and is capable of phosphorylating pyrimidine and purine nucleoside derivatives, as well as deoxothymidine. The discovery that acycloguanosine is phosphorylated by the viral TK but not significantly by the mammalian TKs resulted in one of the most successful approaches to the treatment of herpes simplex virus (HSV) infection and in many gene therapy approaches [9]. HSV1- tk, with specific upstream promoter/enhancer elements, is transinfected into target cells by the selected vector in suicide gene therapy protocols in combination with ganciclovir administration [10].

Inside transinfected cells, the HSV1- tk gene is transcribed to HSV1- tk mRNA and then translated on the ribosomes to HSV1- TK (an enzyme). After administration of a complementary radiolabelled reporter probe and its transport into transduced cells, the 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 (Fig. 1).

Currently, HSV1- tk and a mutant, HSV1- sr39tk, are the most widely used reporter genes in molecular imaging studies that use radiolabelled probes for PET analysis.

The two commonly used radiolabelled reporter probes are [124]FIAU and [18]FHBG. The reporter probe is only used in tracer doses and not in pharmacological doses as in suicide therapy, so there are no known problems concerning cell toxicity.

Several research groups have shown that HSV1- tk can be used as a reporter gene as well as a therapeutic gene. This represents an ideal situation in which the therapeutic and reporter genes are the same [11–14]. Experimental validation of this approach has been demonstrated in animal models of liver colorectal metastases treated with adenovirus-mediated HSV1- tk gene transfer and ganciclovir [15, 16], providing successful gene transduction and an additional margin of safety, because the transduced cells can be easily eliminated by treatment with ganciclovir.

Second reporter gene: dopamine type 2 receptor

$^{18}$F-Fluoroethylspiperone (FESP) is a positron-labelled analogue of the dopamine antagonist spiperone. This tracer was developed to evaluate the level of D2R in vivo in animals and humans. An endogenous high level of D2R expression is limited primarily to the striatum. D2R gene is used as a reporter gene and FESP as a reporter probe for imaging gene expression [17, 18].

The choice of a promoter to drive reporter gene expression depends on the intended application. Strong