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PROSPECTS

In Vivo Imaging and Tumor Therapy With the Sodium Iodide Symporter

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Abstract There has been great progress in the design of vectors for cancer gene therapy. However, it has been difficult to translate success in the laboratory into clinical practice. A major hurdle in understanding these failures has been the relative difficulty in monitoring repeatedly and non-invasively the biodistribution, gene expression and replication of these viral vector systems. With the advent of molecular imaging technology, this deficiency is being rapidly rectified. A number of reporter genes have been used to monitor gene expression. In this review, we discuss the role of the sodium iodide symporter (NIS) as a reporter and therapeutic gene for cancer gene therapy when combined with various radioactive isotopes. J. Cell. Biochem. 90: 1079–1086, 2003. © 2003 Wiley-Liss, Inc.

Key words: gene therapy; NIS; biodistribution

The field of gene therapy has experienced enormous progress over the last decade; several hundred clinical trials have been approved and more than a thousand patients have been enrolled in them. Viral as well as non-viral vectors have been administered through a variety of routes (e.g., intratumoral, intracavitary, and systemically) in attempts to treat a variety of diseases (e.g., metabolic, infectious, and neoplastic). Effective gene therapy depends upon effective expression of the therapeutic gene in the organ/tissue of interest for the desired period of time (short lived for cancer, long term for correction of metabolic diseases). Often, however, these gene therapy trials fail; and it has been very difficult to understand what, where, and why things went wrong. Vectors may fail to reach or enter the target tissue or therapeutic genes may be silenced by intracellular mechanisms. Thus, gene therapists are particularly interested in understanding the location (biodistribution) of the vectors they administer, the efficiency of target tissue transduction, vector population (proliferation and elimination) as well as the level and duration of expression of the therapeutic gene. This implies repeated assessment of these parameters akin to measuring drug levels in clinical pharmacology. Thus it is considered to be advantageous for the field if gene therapy vectors can be engineered to allow the reliable and safe non-invasive monitoring of vector biodistribution and population together with therapeutic gene expression.

Recent technological advances in nuclear medicine, magnetic resonance spectroscopy, optical and bioluminescence imaging have prompted the development of a new field of molecular imaging [Tjuvajev et al., 1995; Gambhir et al., 2000]. Each of the various imaging technologies have their specific advantages and disadvantages, which will not be discussed in any detail in this study. However, all modalities have common features such as the

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use of reporter genes that generate a specific signal that can be detected using the appropriate technology (e.g., gamma camera, single photon emission computerized tomography (SPECT) or PET with gamma photon emitting isotopes; light photon emission with luciferase/D-luciferin). In some situations, the reporter and therapeutic gene are one and the same (e.g., thymidine kinase (tk) and the sodium iodide symporter (NIS)) while in other contexts reporter and therapeutic gene expression are linked either by expression from a single promoter (both genes linked by an internal ribosome entry site or fusion genes) or both genes are expressed from their own separate promoter. In the latter case, proving concordance between expression of the therapeutic and reporter genes is more problematic.

The ideal reporter gene should be non-immunogenic, allows amplification of the signal (high sensitivity), has a limited and well-defined biodistribution and expression in the body, does not interfere with viral kinetics, and the probes necessary for its detection are readily available and preferably approved for clinical purposes. While none of the reporter genes in use so far has the ideal characteristics, we propose that *NIS* comes very close to being the ideal reporter gene. The characteristics of *NIS* and its use as both a reporter and therapeutic gene for cancer therapy will be discussed below.

NIS—STRUCTURE AND PHYSIOLOGICAL FUNCTION

The cDNA for rat [Dai et al., 1996], human [Smanik et al., 1996], and murine NIS [Pinke et al., 2001] has been described in the last few years. Carrasco's group reported the isolation and sequencing of a cDNA from a rat thyroid cell line (FRTL-5) coding for a protein that when ectopically expressed induced active iodide transport in cells [Dai et al., 1996]. The open reading frame of the isolated cDNA suggested that rat NIS was a protein with 618 amino acids with at least 12 membrane spanning domains although further biochemical characterization lead to the revised and now widely accepted view that NIS has 13 membrane spanning domains with the amino terminal end on the extracellular surface of the cell and the carboxy terminal end in the cytoplasm. Three N-linked glycosylation sites have been identified to date (Fig. 1) [Levy et al., 1998]. The gene for human NIS has been localized to chromosome 19p12-13.2 and is composed of 15 exons with the fully processed mRNA coding for a 643 amino acid protein [Smanik et al., 1996, 1997].

NIS is expressed primarily on the basolateral membrane of thyroid epithelial cells [Jhiang et al., 1998]. It is responsible for active iodide uptake in thyrocytes, the first essential step in a series of biochemical changes culminating in the incorporation of the ion within tyrosine residues in thyroglubulin, the precursor for thyroid hormone biosynthesis [De La Vieja et al., 2000]. However, NIS is also expressed, although at lower levels, in many other organs including the salivary and lacrimal glands, stomach, choroid plexus, lactating mammary gland, kidney epithelial cells, and placenta [Jhiang et al., 1998; Spitzweg et al., 1998, 1999a, 2001b; Tazebay et al., 2000]. NIS clearly plays a key role in thyroid hormone biosynthesis by concentrating the relatively rare iodide anion into thyroid cells; the role the protein

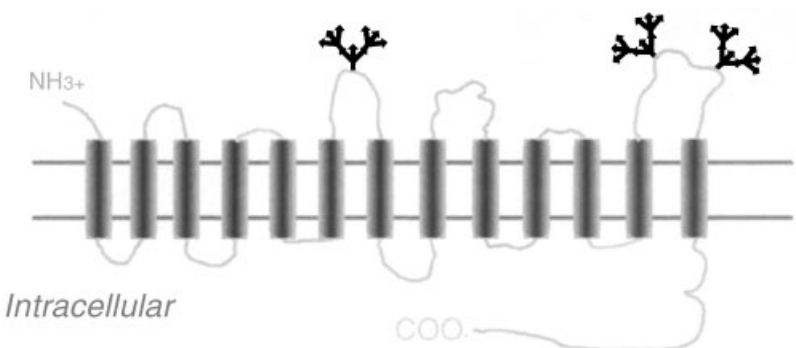

Fig. 1. Schematic representation of the secondary structure of NIS. The protein spans the plasma membrane of cells 13 times and has the amino-terminal end facing the extracellular space and the carboxyterminal end in the cytoplasm. There are three sites for N-linked glycosylation (red).

plays in the other organs where it is expressed is not clear at the present time. In the thyroid NIS expression, subcellular localization, and activity are tightly regulated by a complex interplay of signals including thyroid stimulating hormone (TSH), retinoids, iodide, and various other growth factors [Jhiang, 2000; Riedel et al., 2001]. There is no evidence yet that these mechanisms regulate NIS expression or function in extrathyroidal sites.

Detailed electrophysiological studies of cells expressing NIS show that the symporter transports two sodium ions with one iodide ion across the membrane of cells [Eskandari et al., 1997]. The ubiquitously expressed sodium–potassium ATPase maintains the intracellular sodium concentration at a low level. Coupling of iodide import with sodium uptake (along its electrochemical gradient) drives the active transport of iodide against its electrochemical gradient. In this way, thyroid cells can maintain an iodide concentration that is at least 40-fold higher than that of the extracellular space [De La Vieja et al., 2000]. Ion binding to NIS is non-random; two sodium ions bind first, followed by an iodide ion when the complex presumably undergoes a conformational change that transports the three ions to the cytoplasmic face of the plasma membrane [Eskandari et al., 1997]. NIS can transport into cells many other anions coupled with sodium transport. These include ClO_3^-, SCN^-, $SeCN^-$, NO_3^-, Br^-, TcO_4^-, RhO_4^-, and ^{211}At [Eskandari et al., 1997; Carlin et al., 2002; Van Sande et al., 2003]. There is some controversy regarding the selectivity of NIS for the various anions depending on whether the measurements were made electrophysiologically on cells or on thyroid preparations. Similarly, there is an ongoing debate on the ability of NIS to transport perchlorate into cells or whether perchlorate simply acts as a specific inhibitor of iodide transport by binding to NIS and blocking iodide uptake [Wolff, 1998; De La Vieja et al., 2000; Van Sande et al., 2003].

The availability of so many different isotopes that can be transported by NIS allows the combination of NIS and radioisotopes to achieve specific objectives such as imaging (reporter function) as well as therapy with ionizing radiation emitted during the decay of these isotopes. The pertinent physical characteristics of the most commonly used isotopes are presented in Table I and the relevance of these characteristics is discussed in the subsequent sections.

NIS AS A REPORTER GENE

The field of therapeutic gene delivery for metabolic disorders as well as cancer has seen significant advances due to the availability of an increasing repertoire of vector (viral and non-viral) systems. As our understanding of the regulation of gene expression has increased, vectorologists have incorporated new design features in vector systems not only to enhance the levels of therapeutic gene expression but also to maintain expression for long periods of time. Monitoring of in vivo gene expression is critical for the evaluation of the success or failure of these gene therapy approaches. Tissue sampling might provide some insight into this problem but repeatedly performing biopsies on multiple tissues is inconvenient and some organs are relatively inaccessible (e.g., the brain). Thus, mechanisms for repeated and non-invasive imaging of therapeutic gene expression are considered to be highly desirable. The need for this technology is further highlighted by the advent of replication competent viruses for cancer gene therapy where it is critically important to monitor the biodistribution, replication, expression, and elimination of these viruses in living subjects [Russell, 2002].

TABLE I. Physical Characteristics of Some of the Radioisotopes That may be Used in Combination With NIS

Isotope	Particle emitted	Mean path length (mm)	γ Photon energy (keV)	Physical half-life (days)
^{99m}Tc	e^- (Auger)	<0.001	140	0.25
^{123}I	e^- (Auger)	<0.001	156	0.55
^{124}I	e^+	Variable	511	4.12
^{125}I	e^- (Auger)	<0.001	27–35	60
^{131}I	e^-	0.829	364	8
^{186}Re	e^-	1.8		3.8
^{188}Re	e^-	4.42	155	0.7
^{211}At	e^-	0.04–0.08	77–92	0.3

e^-, electron; e^+, positron.

A number of candidate reporter genes have been evaluated in this field, including thymidine kinase, a mutated dopaminergic receptor (D2), the somatostatin receptor (SSTR) as well as NIS [Tjuvajev et al., 1996; Spitzweg et al., 2000, 2001a; Liang et al., 2001; Ray et al., 2001; Cho et al., 2002; Groot-Wassink et al., 2002]. All these reporter systems depend on expression of a protein interacting with a specific radiolabeled probe that emits a gamma photon signal that can be detected. With the exception of the SSTR, that binds to its substrate with a one to one stiochiometry, all other reporter proteins have the capacity to amplify the signal by trapping the radio-ligand into the cell expressing the specific protein thus enhancing the sensitivity of the reporter system. PET imaging is attractive because it provides high resolution and is more sensitive that gamma camera imaging (by at least a logarithm). In addition, it provides quantitative information about the distribution of the tracer that is immediately translated into concentration of the tracer into the tissue of interest. Thus, PET imaging is highly desirable for dosimetry calculations. Indeed, Groot-Wassink et al. [2002] have utilized NIS in combination with ^{124}I to monitor the biodistribution and expression of a replication incompetent adenovirus using PET imaging. Recently, we were able to monitor the distribution of a replication competent measles virus (MV) engineered to express NIS (MV-NIS) in a myeloma xenograft model using ^{124}I (Dingli et al, article in preparation). ^{124}I is not an ideal radiotracer due to its low positron abundance (25%) and the concomitant emission of high-energy gamma photons that make accurate dosimetric calculations problematic [Pentlow et al., 1991]. In addition, the positrons emitted tend to have relatively high energies and result in lower resolution compared to more conventional isotopes such as ^{18}F. However its long half-life (Table I) allows tracking of slow biochemical processes and a cyclotron need not be on site for synthesis of the isotope.

Gamma camera imaging can provide adequate data for monitoring and quantification of in vivo gene expression as well as dosimetric calculations [Spitzweg et al., 2000, 2001a; Cho et al., 2002; Dingli et al., 2003]. We have previously shown that serial imaging of mice bearing tumor xenografts expressing NIS and injected with ^{123}I can be used to estimate the energy absorbed by tumor xenografts that were eventually successfully treated with ^{131}I [Spitzweg et al., 2000, 2001a; Dingli et al., 2003a].

NIS expression technology can also be used for serial imaging of replication competent vectors. Recombinant, replication competent MVs based on the Edmonston vaccine strain of MV (MV-Edm) are potently and selectively oncolytic [Peng et al., 2001, 2002]. MV-Edm eliminates myeloma tumor xenografts composed of KAS-6/1 or ARH-77 cells by inducing cell to cell fusion with the formation of giant cell syncytia. The *NIS* gene was cloned into a recombinant infectious molecular clone of MV-Edm to generate MV-NIS. The recombinant virus was rescued and shown to induce expression of NIS in tumor cells. To study the distribution and expression of this vector, SCID mice bearing KAS-6/1 tumor xenografts were injected intravenously with MV-NIS (Fig. 2).

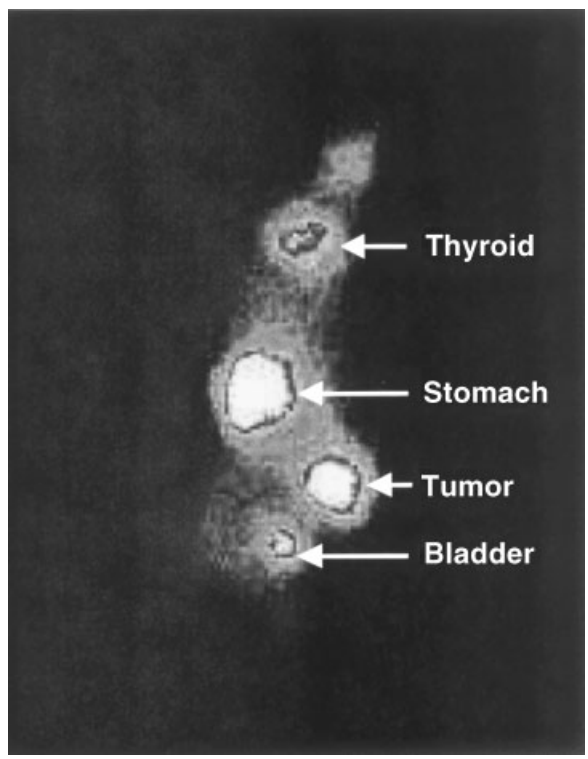

Fig. 2. In vivo imaging of viral gene expression in an animal tumor model. Gamma camera scintiscan of a mouse bearing a myeloma tumor xenograft in the right flank. The mouse was injected intravenously with MV-NIS and imaged 9 days later after the administration of ^{123}I (18.7 MBq, i.p.). The image was acquired over 5 min using a conventional gamma camera. Physiologic NIS expression and iodide uptake are seen in the salivary glands, the thyroid, and the stomach. The isotope is filtered by the kidneys and accumulates in the bladder.

The mice were serially imaged after repeated ^{123}I administration (18.5 MBq intraperitoneally) and a signal from the tumors could be detected as early as 3 days after virus administration. Iodide uptake in the tumors increased upto 9 days after virus injection demonstrating increasing NIS expression as the virus proliferated and infected adjacent tumor cells. By day 17, iodide uptake in the tumors had decreased substantially coincident with the elimination of the tumors by continued viral proliferation [Dingli et al., 2003b].

Routine gamma camera resolution is in the range of 5–10 mm and this is determined mainly by the collimator resolution. While a pin-hole collimator improves image resolution, this requires prolonged image acquisition times with the risk of motion artifact [Cho et al., 2002]. We routinely image our animals using a standard gamma camera with a parallel collimator and obtain good quality images after a 5 min exposure. Resolution can be improved by the use of SPECT, although this requires longer image acquisition times and correction for both motion artifact and signal attenuation. With the availability of advanced, semiconductor based detector systems, gamma camera resolution should improve and may even allow simultaneous imaging with multiple isotopes emitting photons of different energies.

While no head to head comparisons have been published to date, we believe that NIS has several potential advantages over the other reporter systems. NIS is a physiologically expressed protein that only rarely induces the formation of antibodies while tk from the human herpes viruses is theoretically immunogenic [Morris et al., 1997]. The tracers used in combination with NIS (^{99m}Tc or iodide isotopes) are readily available commercially at a relatively low cost. In contrast, the radiotracers required for PET imaging require not only the availability of a cyclotron on site due to the short half-life of the isotope (^{18}F) but also a sophisticated radiochemistry laboratory with the necessary facilities for synthesis of the radiotracers. In addition, gamma camera technology is much more widely available compared to the expensive PET scanners. Finally, iodide and pertechnetate are already approved by the Food and Drug Administration for clinical applications; thus it is logistically much simpler to translate their use into clinical practice.

NIS AS A THERAPEUTIC GENE FOR CANCER

Advanced but well differentiated thyroid cancer has been successfully treated for more than 50 years with radioiodine (^{131}I) [Mazzaferri and Kloos, 2001]. These tumors can concentrate and retain radioiodide that decays by the emission of an electron (β particle). The emitted electrons induce direct damage to cellular DNA and other subcellular compartments such as mitochondria as well as the generation of highly reactive free radicals that ultimately lead to cell death [Ferlini et al., 2002]. With the availability of the cDNA for NIS, various groups reported that NIS gene transfer in different tumor models results in significant iodide concentration both in vitro as well as in vivo [Mandell et al., 1999; Spitzweg et al., 1999b, 2000, 2001a; Nakamoto et al., 2000; Carlin et al., 2002; Cho et al., 2002; Dingli et al., 2003a]. Iodide uptake can be blocked by perchlorate, a specific inhibitor of NIS or by blocking the sodium–potassium ATPase. The administration of a single dose of ^{131}I to mice bearing prostate carcinoma and myeloma tumor xenografts led to complete and long-lasting tumor eradication with minimal toxicity [Spitzweg et al., 2000, 2001a; Dingli et al., 2003a].

Patients with disseminated malignancies often have an extensive tumor burden and it is probably not possible to transduce every single tumor cell with any of the vectors currently available for cancer gene therapy. It is, therefore, considered desirable to have therapeutic genes with a bystander effect, whereby non-transduced tumor cells are still eradicated by the therapy. In this respect, the combination of NIS and radioisotopes has a potential advantage over other therapeutic genes since the electrons emitted from either ^{131}I, ^{186}Re, or ^{188}Re have a macroscopic path length that is measured in millimeters (Table I). Thus, NIS expression by a small proportion of tumor cells with resultant iodide uptake can lead to destruction of surrounding tumor cells that escaped transduction by the vector. These cells will be caught in the electron cross-fire emanating from the transduced cells leading to their elimination. In a series of tumor mixing experiments, we showed that NIS and radioiodine (^{131}I) have a bystander effect. Myeloma tumor xenografts, composed of mixed populations of cells transduced to express NIS (ARH-NIS) and

the parental cell line ARH-77, were implanted in SCID mice. The proportion of cells expressing NIS in these tumors varied from 10% to 100%. NIS could be detected in all tumor xenografts even when only 10% of the cells expressed the protein and therapy with ^{131}I led to the eradication of tumors with only half of the cells expressing NIS. The growth of tumors that had only a small fraction of cells expressing NIS (10%) was transiently but significantly slowed down [Dingli et al., 2003]. Similarly, other groups have reported on in vitro data demonstrating that the therapeutic effect of NIS and ^{131}I is enhanced when tumor cells are grown as spheroids compared to a monolayer. Tumor geometry enhances the therapeutic effect by maximizing particle cross fire with maximal energy deposition in the tumor for any given dose of isotope [Carlin et al., 2000; Mitrofanova et al., 2003].

If one were to ignore the variable sensitivity of tumors to radiation, isotope retention by the tumor is a major determinant of the outcome of therapy. Radioisotopes must not only be concentrated but also retained for sufficient time within the tumor to allow enough disintegrations to occur thus ensuring tumor eradication. Tumor cells induced to express NIS by gene transfer leak out iodide via anion channels and possibly also through NIS, although this is not very likely due to the high extracellular sodium ion concentration [Mandell et al., 1999; Nakamoto et al., 2000]. However, various investigators have reported on the success of radioiodine therapy after NIS gene transfer as discussed above. Nakamoto et al. [2000] and our own observations suggest that tumor cells expressing NIS leak out iodide at a slower rate compared to thyroid cell lines. Indeed, at high levels of NIS expression, iodide efflux from tumor cells follows zero order kinetics and is independent of intracellular iodide concentration. In addition, there is significant iodide reuptake by tumor cells expressing NIS. In this respect tumor geometry plays an important role in determining the duration of isotope retention in the vicinity of the tumor and, therefore, the therapeutic outcome (Dingli et al., article submitted).

If tumor cells do not express high levels of NIS or if transduction efficiency is not high, it might be possible to eradicate the tumors by treating them with ^{186}Re, ^{188}Re (both as perrhenate), or with ^{211}At. The rhenium isotopes emit higher energy electrons and have shorter half-lives compared to ^{131}I (Table I). Therefore, for any given time that these isotopes are retained by the tumor, more disintegrations will occur within the environment of the tumor from perrhenate compared to iodide and the potential bystander effect is higher due to the longer path length (Table I). Gamma photons emitted by ^{188}Re are almost ideal for gamma camera imaging allowing simultaneous imaging of isotope and vector distribution. The alpha particles emitted by ^{211}At decay are potently ionizing and can damage cells independent of oxygen tensions (unlike electrons). Thus, astatide in combination with NIS gene transfer has the potential for both significant tumor cytotoxicity (even under hypoxic conditions) as well as a bystander effect. While the short half-life of the isotope is a potential disadvantage since it has to be manufactured on site in a cyclotron, it can also enhance its therapeutic effect by rapid decay and maximal energy delivery within the tumor. Reports on the successful use of these isotopes in combination with NIS gene transfer for tumor eradication are eagerly awaited.

CONCLUDING REMARKS

The field of gene therapy has made considerable strides in the last decade by the development of new vectors and an increasing repertoire of therapeutic genes. Monitoring the in vivo distribution, replication, and elimination of replicating vectors as well as therapeutic gene expression have been recognized as critical elements in the design of clinical trials to understand the outcomes of gene therapy studies. With the advent of sophisticated imaging technology based on the use of radiotracers, optical or paramagnetic properties, these aims have been achieved in animal models and should be translated in the clinic in the foreseeable future. NIS gene transfer combines both the potential for in vivo imaging as well as therapy for various radiosensitive malignancies. The gene can be used as a simple reporter when it is expressed as part of a bicistronic transcript or as a therapeutic gene when combined with the various isotopes mentioned above. In this respect, NIS allows personalized therapy with radioisotopes since pre-therapy dosimetric calculations can allow very accurate estimations of the amount of radioisotope necessary to deliver a given radiation dose to a tumor.

The availability of radiochemicals that are already clinically approved should speed the transfer of this technology from the laboratory to the clinic.

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