The Na^+/I^- Symporter (NIS): Imaging and Therapeutic Applications

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The Na^+/I^- symporter (NIS) is the plasma membrane glycoprotein that mediates the active uptake of I^- in the thyroid, ie, the crucial first step in thyroid hormone biosynthesis. NIS also mediates I^- uptake in other tissues, such as salivary glands, gastric mucosa, and lactating (but not nonlactating) mammary gland. The ability of thyroid cancer cells to actively transport I^- via NIS provides a unique and effective delivery system to detect and target these cells for destruction with therapeutic doses of radioiodide. Breast cancer is the only malignancy other than thyroid cancer to have been shown to functionally express NIS endogenously. The considerable potential diagnostic and therapeutic use of radioiodide in breast cancer is currently being assessed. On the other hand, exogenous NIS gene transfer has successfully been carried out into a variety of other cell lines and tumors, including A375 human melanoma tumors, and SiHa cervix cancer, human glioma, and hepatoma cell lines. Most notably, significant radioiodine therapy results have been obtained in the NIS-transfected human prostatic adenocarcinoma cell line LNCaP and in NIS-transfected myeloma cells, both of which exhibited prolonged retention of radioiodide even in the absence of I^- organification. The therapeutic potential of alternative NIS-transported radioisotopes with different decay properties and a shorter, physical half-life than $^{131}I^-$, such as β-emitter ^{188}Rhenium ($^{188}ReO_4^-$) and α-emitter ^{211}Astatine ($^{211}At^-$), has been evaluated. In conclusion, it is clear that the remarkable progress made in the last few years in the molecular characterization of NIS has created new opportunities for the development of diagnostic and therapeutic applications for NIS in nuclear medicine.

THE Na^+/I^- symporter (NIS) is an integral plasma membrane glycoprotein most commonly studied in connection with the thyroid gland, where NIS mediates the active transport of I^- into the thyroid follicular cells as the crucial first step in thyroid hormone biosynthesis. Thyroid hormones T_3 and T_4 (tri-iodothyronine and thyroxine [or tetra-iodothyronine], respectively) are the only iodine-containing hormones in vertebrates. Because I^- is an essential constituent of T_3 and T_4, both thyroid function and its systemic ramifications depend on an adequate supply of I^- to the gland.[1] This supply in turn depends on the sufficient dietary intake of I^- and proper NIS function. NIS is also expressed endogenously functionally in other tissues, including salivary glands, gastric mucosa, and lactating mammary gland, in all of which NIS mediates active I^- transport. While the functional significance of NIS in the gastric mucosa and salivary glands is unknown, in the lactating mammary gland, NIS mediates the translocation of I^- into the milk, making this anion available for the nursing newborn to biosynthesize his/her own thyroid hormones.[2,3]

The ability of the thyroid to accumulate I^- via NIS has long provided the basis for diagnostic scintigraphic imaging of the thyroid with radioiodide. It has served as an effective means for therapeutic doses of radioiodide to target and destroy hyperfunctioning thyroid tissue, such as in Graves' disease, and to destroy I^--transporting thyroid carcinoma cells, both within primary tumor remnants after surgery and in metastases.[4] Therefore, the study of NIS is of high relevance to thyroid pathophysiology. Nevertheless, no molecular information on NIS was available until very recently. In the absence of nucleotide and protein sequence information, the detailed molecular characterization of NIS started in 1996 when the complimentary deoxyribonucleic acid (cDNA) encoding rat NIS was isolated by Dai and coworkers by expression cloning in *Xenopus laevis* oocytes, using cDNA libraries derived from a highly functional rat thyroid-derived cell line (FRTL-5 cells).[5]

Another major development in the molecular characterization of NIS was the generation of high affinity ($K_d \sim 1$ nM), site-directed, polyclonal anti-NIS Abs against the carboxyl terminus of the protein, by Levy and coworkers.[6] Based on the cloned cDNA, rat NIS was determined to be a protein of 618 amino acids (relative molecular mass 65,196). The current secondary structure model for NIS, based on extensive experimental testing, proposes 13 transmembrane segments, with the amino terminus facing extracellularly and

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the carboxyl terminus intracellularly (Fig 1).[7] The cDNA encoding human NIS (hNIS) was subsequently identified on the expectation that hNIS would be highly homologous to rat NIS (rNIS). Using primers to the cDNA rNIS sequence, Smanik and coworkers identified a cDNA clone encoding hNIS.[8] The nucleotide sequence of hNIS revealed an open reading frame of 1,929 nucleotides, which encodes a protein of 643 amino acids. The hNIS shows an 83% identity and 93% similarity to rNIS.

In all of the tissues and cells where it is functionally expressed, NIS couples the inward "downhill" translocation of Na^+ to the inward "uphill" translocation of I^-, establishing ~20-fold to 40-fold I^- concentration gradients under steady-state conditions. The driving force for the process is the inwardly directed Na^+ gradient generated by the Na^+/K^+ adenosinetriphosphatase (ATPase). NIS activity in all these cells is blocked by the well-known, "classic" competitive inhibitors, the anions thiocyanate (SCN^-) and perchlorate (ClO_4^-). In thyroid follicular cells, NIS mediated I^- accumulation is stimulated by thyroid-stimulating hormone (TSH). I^- is then translocated from the cytoplasm of these cells across the apical plasma membrane towards the colloid in a process called I^- efflux, mediated by a different transporter that has yet to be identified unequivocally.[9,10] In a complex reaction called organification of I^-, catalyzed by thyroid peroxidase (TPO) at the cell-colloid interface, I^- is oxidized and incorporated into some tyrosyl residues within the thyroglobulin (Tg) molecule, leading to the subsequent coupling of iodotyrosine residues. The term organification refers to the covalent incorporation of I^- into organic molecules (in this case Tg), as opposed to nonincorporated, inorganic, or free I^-. I^- organification in the thyroid results in the retention and storage of I^- within the gland. The I^- organification reaction can be blocked pharmacologically by 6-*n*-propyl-2-thiouracil and 1-methyl-2-mercaptoimidazole. In response to the demand for thyroid hormones, phagolysosomal hydrolysis of endocytosed iodinated Tg ensues. T_3 and T_4 are secreted into the bloodstream. All of these steps, like NIS-mediated I^- uptake, are stimulated by TSH. In contrast, NIS in extrathyroidal tissues is not subjected to regulation by TSH, and these tissues show minimal or no I^- organification.[2,3]

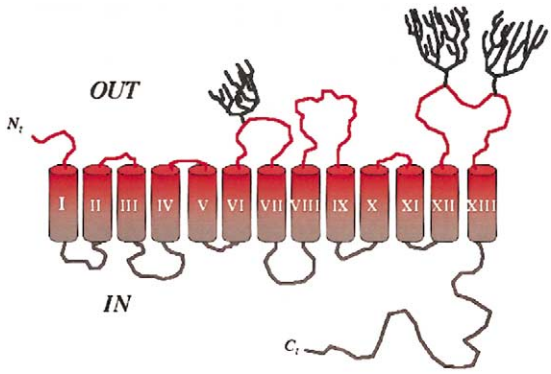

Fig 1. Secondary structure model of Na+/I^- symporter (NIS). Structure contains 13 putative transmembrane segments (TMS). Both the hydrophilic loop containing N225 and the NH_2 terminus face extracellularly. All 3 N-linked glycosylation consensus sequences are indicated at positions 225, 485, and 497. Reprinted with permission.[2] (Color version of figure is available online.)

ENDOGENOUS VERSUS EXOGENOUS NIS EXPRESSION IN CANCER: ENDOGENOUS IN THYROID AND BREAST CANCER, EXOGENOUS ELSEWHERE

Endogenous NIS, as indicated previously, is physiologically and functionally expressed in only a few normal tissues, including salivary glands, gastric mucosa, and lactating mammary gland. The only cancer known for decades to express endogenous NIS was thyroid cancer. The ability of cancerous thyroid cells to transport actively I^- via NIS provides a unique and effective delivery system to detect and target these cells for destruction with therapeutic doses of radioiodide, largely without harming other tissues. Therefore, it seems feasible that radioiodide could be a diagnostic and therapeutic tool for the detection and destruction of other cancers in which endogenous NIS is functionally expressed. Pointing in this direction is a report by Tazebay and coworkers in which they showed that human breast carcinomas and experimental mammary carcinomas in transgenic mice express NIS.[11] In vivo scintigraphic imaging of experimental mammary adenocarcinomas in nongestational and nonlactating female transgenic mice carrying either an activated *ras* oncogene (*c-Ha-ras*) or overexpressing the *Neu* oncogene (*c-erbB-2*) showed pronounced, active, specific, and ClO_4^--inhibitable NIS activity. This was also observed later in transgenic mice overexpressing the polyoma middle T antigen (PyV). Clearly, transgenic mice bearing experimental mammary

tumors provide an excellent model to study the potential role of endogenous NIS expression in mammary cancer and, particularly, the possible effectiveness of radioiodide therapy in combating this disease.

By immunohistochemistry, Tazebay and coworkers showed further that 87% of 23 human invasive breast cancers and 83% of 6 ductal carcinomas in situ expressed NIS, as compared with only 23% of 13 extratumoral samples from the vicinity of the tumors.[11] Even more significantly, none of the 8 normal samples from reductive mammaplasties they studied expressed NIS. Kogai and colleagues reported an increase in NIS messenger ribonucleic acid (mRNA), NIS protein, and I^- uptake activity in a human mammary adenocarcinoma cell line (MCF-7) in response to transretinoic acid treatment.[12] Dohán and coworkers have recently developed a method for early detection (by flow cytometry) of NIS expression in human mammary adenocarcinoma cells collected by fine needle aspiration.[3] The results obtained with this method correlate closely with NIS expression detected by immunohistochemistry of the corresponding biopsy specimens.

Wapnir and coworkers examined the immunohistochemical profile of NIS in thyroid, breast, and other carcinomas using high-density tissue microarrays and conventional sections.[13] Validating the use of microarrays, the study showed that results obtained with microarrays, which allow the simultaneous analysis of multiple samples on the same slide, correlated closely with those obtained with conventional sections. Insofar as breast in particular was concerned, they analyzed as many as 371 breast specimens, and the results confirmed the findings stated previously: NIS expression was observed in whole tissue sections in 76% of invasive breast carcinoma and 88% of ductal carcinoma in situ samples. The majority of normal breast cores were negative (87%), as were 70% of normal/nonproliferative samples analyzed. Plasma membrane immunoreactivity was observed in gestational breast tissues, and in some in situ ductal carcinomas and invasive ductal carcinomas. Because these studies in human samples were performed by immunohistochemistry, the findings show only endogenous NIS expression but not necessarily endogenous NIS *functional* expression in human breast cancer.

Addressing this issue, Moon and coworkers have reported pertechnetate ($^{99m}TcO_4^-$) accumulation in primary breast tumors in humans in vivo.[14] As discussed extensively later, $^{99m}TcO_4^-$ is widely used for diagnostic imaging and is also transported by NIS, with the advantage of having a shorter half-life (6 hours) than ^{131}I (8 days). These investigators studied 25 patients with cancer by scintigraphy and found active uptake by the tumors in 4. This is a highly meaningful result, not only because it shows the existence of I^- transport activity in a significant percentage of studied human patients with breast cancer in vivo, but also because the observation was made in patients whose thyroids were not downregulated (ie, thyroid NIS was still expressed, and, therefore, there was avid thyroidal $^{99m}TcO_4^-$ uptake; this decreased the amount of radioisotope available for uptake by breast tumors). It is possible that a larger proportion of $^{99m}TcO_4^-$-accumulating breast cancer tumors might have been detected by scintigraphy if the availability of the radioisotope to tumoral tissue had been optimized by thyroid suppression.

In conclusion, although more extensive studies in humans are still necessary, functional expression of endogenous NIS in breast cancer has been documented in both experimental mice and humans. This result strongly suggests that NIS is upregulated with high frequency during malignant transformation in breast cancer. Therefore, only 2 cancers, those of the thyroid and the breast, have been shown to express endogenous NIS functionally. The tremendous potential of this finding for the use of radioiodide in the diagnosis and treatment of breast cancer remains to be fully assessed. For other cancers, exogenous NIS gene transfer is actively being researched.

EXOGENOUS NIS GENE TRANSFER FOR IMAGING

The cloning and characterization of NIS, in light of the ample experience accumulated over the last 60 years of imaging and treating thyroid patients with radioiodide, has led to the development of a novel gene transfer strategy in other tissues: the targeted expression of functional exogenous NIS in selected cells aimed at rendering them susceptible to imaging and/or destruction with radioiodide. Several in vitro experiments on NIS gene transfer for diagnostic and therapeutic purposes have been reported in which NIS-mediated radioiodide up-

take was used to visualize and destroy malignant tumor cells. The NIS gene has clear and compelling advantages as a reporter gene. Scintigraphic imaging of NIS expression can easily be attained with commercially available, inexpensive radionuclide probes, such as $^{131}I^-$, $^{123}I^-$, and $^{99m}TcO_4^-$, all of which have long been approved for human use. Groot-Wassink and coworkers have studied the ectopic expression of the hNIS gene in vivo and monitored it in biodistribution studies on intravenous injection of $^{125}I^-$.[15] Adenovirus (Ad) delivery successfully induced NIS gene expression in the liver, adrenal glands, lungs, pancreas, and spleen. In addition, NIS expression was also readily detected in tumor xenograft models when the virus was injected intratumorally. Finally, NIS expression was monitored by positron emission tomography (PET) after intravenous injection of $^{124}I^-$, showing the potential of this approach for noninvasive imaging.

Although NIS can conceivably serve as a pure reporter gene for noninvasive imaging of the transfer of other therapeutic transgenes, most efforts have concentrated on using NIS for imaging of its own expression in selected transfected cells or tumors, with the purpose of quantifying NIS expression and establishing its expression efficiency for follow-up radioiodine therapy. In one of the first publications on NIS gene transfer for imaging purposes, Shimura and coworkers reported transfection of the rNIS cDNA in malignantly transformed rat thyroid cells (FRTL-Tc) that ordinarily do not show I^- transport activity.[16] The cell line that resulted from the transfection (ie, Tc-rNIS) displayed a 60-fold increase in $^{125}I^-$ accumulation over background in vitro. Tumors formed with Tc-rNIS cells accumulated up to 27.3% of the total administered $^{125}I^-$, showing an 11-fold to 27-fold increase in $^{125}I^-$ concentration, as compared with nontransfected cells. Mandell and colleagues scintigraphically imaged rNIS-transduced A375 human melanoma tumors, which accumulated significantly more $^{123}I^-$ than non-transduced tumors.[17]

For their part, Boland and coworkers used the AdNIS vector to transfect human tumor cells, namely SiHa cervix cancer cells and MCF-7 breast cancer cells, in nude mice.[18] A quantitative analysis revealed that uptake in AdNIS-injected tumors was 4 to 25 times higher than in nontreated tumors. On average, 11% of the total amount of injected $^{125}I^-$ was recovered per gram of AdNIS-treated tumor tissue. Cho and colleagues showed functional expression of hNIS in a xenografted human glioma as a result of intratumoral injection of recombinant adenovirus rAd-CMV-hNIS.[19] Nakamoto and coworkers established a novel breast cancer cell line, MCF3B, by stably transfecting NIS into the widely used MCF-7 cells.[20] In a biodistribution study using MCF3B-xenografted mice, high $^{125}I^-$ uptake (16.7%) was observed in the tumors 1 hour after injection. In addition, high tumor-to-normal tissue ratios were also observed (ranging from 4 to 21), except in the stomach (0.47). Sieger and colleagues showed radioiodine uptake in a hepatoma cell line in vitro and in vivo after transfer of the hNIS gene under the control of a tumor-specific regulatory element, the promoter of the glucose transporter 1 gene (GTI-1.3).[21] The same group (ie, Haberkorn and coworkers[22]) used NIS-transduced prostate carcinoma cells to study in vivo biodistribution. In rat, the hNIS-expressing tumors accumulated up to 22 times more I^- than contralateral transplanted wild-type tumors. In conclusion, an impressive body of evidence has already been produced showing the induction of NIS functional expression in nonthyroid tumors or its restoration in undifferentiated thyroid malignancies on the application of gene transfer techniques of the NIS gene.

Exogenous NIS for Therapy

The successful induction of NIS functional expression by NIS gene transfer in nonthyroid and undifferentiated thyroid tumors prompted many groups to study the effects of $^{131}I^-$ therapy in these tumors.[16,18,20,22] However, no tumor shrinkage was detected with treatment, apparently as a result of the rapid radioisotope efflux observed. It appears that as the retention time of $^{131}I^-$ in the tumors was only a few hours, it was not enough to deliver the radiation dose necessary to have a discernible therapeutic effect. Moreover, under these conditions, it is likely that the 8-day half-life and decay properties of $^{131}I^-$, which result in the emission of low-energy ($E_{average}$ = 0.134 MeV) β-particles, also may have played a role in the disappointing results obtained.

Hence, several different approaches have been pursued to circumvent the problem of insufficient radiation dose to NIS-expressing tumors. Boland and coworkers proposed to improve the efficiency of NIS gene transfer and, thus, the I^- uptake

capacity of the target tissue, by using modified vectors and/or higher viral doses.[23] However, it remains to be seen whether this approach will prove safe and will result in increased radiation doses to the tumors. Nakamoto and coworkers[20] and Daniels and Haber[24] have suggested that the fast radioiodine efflux from breast cancer cells might be pharmacologically modulated by administering lithium salts, which increase the half-life of radioiodide. Yet, in vitro experiments with NIS-transduced hepatoma cells performed by Sieger and colleagues[21] revealed that lithium had no significant effect on I^- efflux.

Boland and coworkers have proposed to increase the retention time of radioiodine in tumor cells by coupling the transfer of the NIS gene with the delivery of a gene involved in the I^- organification process, such as TPO.[23] As explained previously, the thyroid is the only tissue known to organify I^- to a significant extent. I^- organification is the covalent incorporation of I^- into selected tyrosyl residues on the Tg molecule.[25] The organification process causes administered radioiodine to be retained within the gland for several days.[26] This relatively long retention time, which matches the physical half-life of ^{131}I (ie, 8 days), allows a significant radiation dose to be delivered to the tissue. However, the simultaneous transfer of both the NIS and TPO genes is not easy to accomplish because of the inherent complexity of gene therapy procedures and the difficulty in transfecting only the desired target tissue (ie, the tumor) in vivo.

Huang and coworkers observed that, although the expression of NIS resulted in significant radioiodide uptake in transfected non-small cell lung cancer (NSCLC) cell lines, rapid radioiodide efflux limited tumor cell death.[27] The transfection of NSCLC cells with human NIS and TPO genes led to increases in radioiodide uptake and retention, as well as to enhanced tumor cell apoptosis. Therefore, the investigators concluded that although single gene therapy with the NIS gene alone may have limited efficacy because of rapid radioiodide efflux, the codelivery of the TPO and NIS genes may be an effective way to circumvent the problem. Boland and coworkers constructed a recombinant adenovirus encoding the human TPO gene under the control of the cytomegalovirus early promoter (AdTPO).[23] Infection of SiHa human cervix tumor cells with this virus led to production of an enzymatically active protein. A significant increase in I^- organification was observed in cells coinfected with AdNIS and AdTPO in the presence of exogenous hydrogen peroxide. However, the levels of I^- organification obtained were too low to increase significantly the I^- retention time in the target cells.

Smit and coworkers studied whether transfection of hNIS into the hNIS-deficient follicular thyroid carcinoma cell line FTC133 makes these cells susceptible to radioiodine therapy.[28] In addition, the effects of a low I^- diet and thyroid ablation on I^- kinetics were investigated. Tumors derived from NIS-transfected FTC133-NIS30 in mice kept on a normal diet showed a high I^- accumulation rate (17.4% of administered activity versus 4.6% in nontransfected tumors). The I^- retention time (ie, I^- biological half-life) in FTC133-NIS30 tumors was 3.8 hours. In mice kept on a low I^- diet, peak activity in FTC133-NIS30 tumors was diminished (8.1%), while I^- accumulation in the thyroid gland was increased. In thyroid-ablated mice kept on a low I^- diet, the half-life of radioiodide was increased considerably (26.3 hours), leading to a much higher area under the time-radioactivity curve than in FTC133-NIS30 tumors in mice on a normal diet without thyroid ablation. Experimental radiotherapy with 2 mCi in thyroid-ablated nude mice kept on a low I^- diet postponed tumor development for up to 4 weeks after therapy, although the tumors eventually regrew. The investigators concluded that the short half-life of I^- in NIS-transfected tumors could only be partially improved by conventional conditioning with thyroid ablation and low I^- diet.

Cho and coworkers have extended their studies on the imaging of NIS-transduced glioma tumors to the analysis of the effect of radioiodine therapy on these tumors.[29] Gliomas are known to be aggressive and radioresistant tumors. Three doses of 4 mCi $^{131}I^-$ were administered to rats bearing hNIS-transduced F98 glioma tumors. Some increase in survival was observed, with the average survival time for the animals with vector alone-transduced F98/LXSN tumors with $^{131}I^-$ treatment, F98/hNIS tumors without $^{131}I^-$ treatment, and F98/hNIS tumors with $^{131}I^-$ treatment being 30.4 ± 3.2, 39.0 ± 4.1, and 45 ± 8.6 days, respectively. Tumor volume seemed to be reduced for a certain period by $^{131}I^-$ treatment, but the tumors eventually re-grew.

Significantly, NIS-transfected cell lines and tumors generated in animal models with these cells

Table 1. Decay Properties of Radioisotopes Used in NIS Research

Radioisotope	Half-Life	Therapeutic Emissions	Photon Energy (keV) and Abundance (%)
^{131}-Iodine	8 d	β^-, E_{av} = 0.134 MeV	364 (81)
^{99m}-Technetium	6 h	none	140 (89)
^{188}-Rhenium	16.9 h	β^-, E_{av} = 0.764 MeV	155 (15)
^{211}-Astatine	7.2 h	α, E = 5.87 MeV	79 (21)

have an ability to retain I^- in significant amounts for sufficient periods without organification. For example, no I^- organification occurs in either the MCF-7 or T-47D mammary tumor cell lines, but the I^- retention time in both of these cell lines is longer than in cells from other origins.[30,18] This result suggests that the I^- efflux system is less efficient or more rapidly saturated in some cell types than in others. In turn, this could explain the encouraging therapeutic results reported by several groups in various NIS-transduced tumors that do not organify I^-. The first reports of this kind were published by Spitzweg and coworkers, who obtained excellent therapeutic results by stably transfecting the human prostatic adenocarcinoma cell line LNCaP with the NIS cDNA under the control of the prostate-specific antigen promoter.[31,32] NIS-transfected NP-1 tumor xenografts in mice accumulated 25% to 30% of the total administered I^-, showing a long retention time (45 hours) of the radioisotope. After administration of a single 3 mCi dose of ^{131}I, significant tumor reduction was achieved in NP-1 tumors in comparison with nontreated controls. It would have been informative to include biodistribution and dose-escalation studies in this report to clarify the investigators' reason for using such a high dose.

The same group recently has recently reported $^{131}I^-$ therapy of NIS-transfected myeloma in SCID mice.[33] They used self-inactivating lentivector with enhanced green fluorescent protein expression under the control of a minimal immunoglobulin promoter to transduce hNIS into myeloma cells. Tumor xenografts in severe combined immunodeficiency mice expressing hNIS were imaged with $^{123}I^-$ and shown to retain 4% to 5% of I^- for up to 48 hours. Based on biodistribution results, the therapeutic dose of $^{131}I^-$ was calculated to be 1 mCi. A single administration of this dose completely eradicated tumor xenografts without evidence of recurrence for up to 5 months after therapy. Although myeloma is inherently a very radiosensitive malignancy, the success of this therapy in the absence of I^- organification is nevertheless very impressive. Noting that treatment was successful even though hNIS was not transduced into every myeloma cell, the investigators explained that nontransduced cells could be killed by electrons emitted by $^{131}I^-$ from distant cells. Such emitted electrons can travel several cell diameters in vivo. This is a classical example of "cross-fire" radiation damage by β-emitters, such as $^{131}I^-$, which was referred to in the article as the "bystander effect."[33]

Alternative NIS-Transported Radioisotopes

In light of the relatively short biological retention time of $^{131}I^-$ in NIS-expressing tumors, alternative NIS-transported radioisotopes with superior decay properties and a shorter physical half-life than $^{131}I^-$ (Table 1) must be considered as potentially better therapeutic options. Two isotopes have been proposed for this purpose, β-emitter ^{188}Rhenium ($^{188}Re^-$) and α-emitter ^{211}Astatine ($^{211}At^-$) (Table 1). It has long been recognized by nuclear medicine practitioners that due to their common ionic characteristics, I^- and ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) behave similarly following intravenous administration.[34] In humans, $^{99m}TcO_4^-$ localizes in the thyroid, salivary glands, gastric mucosa, and choroid plexus of the brain, just like I^-. $^{99m}TcO_4^-$ is concentrated but not organified in the thyroid gland and is used in nuclear medicine as an alternative to $Na^{131}I$ for assessing thyroid conditions. Rhenium (Re) is a chemical analogue of technetium (Tc) and shows practically identical chemical and biodistribution properties to those of Tc.[35]

The perrhenate anion (ReO_4^-) is concentrated in the thyroid and stomach by endogenous NIS because of its chemical similarity to pertechnetate.[36] Dadachova and coworkers have recently proposed the use of ^{188}Re for the treatment of NIS-expressing tumors.[37] The ^{188}Re is a powerful β-emitting radionuclide with a 16.7-hour half-life, and it is conveniently obtained from a $^{188}W/^{188}Re$ generator.[38] The emission characteristics and physical properties of ^{188}Re are superior to those of ^{131}I. The ^{188}Re higher energy β particles (E_{av} = 0.764 MeV versus 0.134 MeV for

^{131}I) are effective over a higher range, sufficient to eradicate medium or large tumors by a "cross-fire" effect,[39] while its lower energy and low abundance gamma photons (155 keV, 15% abundance) are suitable for imaging yet easier to shield than the 364 keV photons of ^{131}I. As a consequence of its emission characteristics, the optimal tissue range for ^{188}Re is 23 to 32 mm, which means that ^{188}Re can be used for the treatment of relatively large solid tumors. The number of atoms of the radioisotope per gram of tumor needed to produce a cure probability of 90% at the optimal range is 6.38×10^{12} for ^{131}I and 5.34×10^{11} for ^{188}Re.[39] These figures clearly show that ^{188}Re would have the potential to deliver larger doses of radiation to tumors expressing NIS than $^{131}I^-$, even if the uptake of ^{188}Re-perrhenate were significantly lower than that of $^{131}I^-$. Our dosimetry calculations indicate that in the absence of organification, ^{188}Re-perrhenate delivers a 4.5-fold higher dose to a 2-g tumor in a human than ^{131}I. Administration of ^{188}Re-perrhenate is safe for the thyroid and stomach in mice,[40] and preliminary encouraging therapy results have been reported in hNIS-transfected hepatocellular carcinoma cells[41] and in hNIS-expressing F98 glioma tumors in animals.[42]

The ^{211}At is an α-emitting radiohalide, and its very high linear energy transfer of 98.8 keV/μm makes its relative biological efficiency close to maximal. The drawbacks of this isotope are its scarce availability (ie, a cyclotron with a deutron beam is needed for ^{211}At production and its short physical half-life of 7 hours precludes long-distance transportation) and nontrivial safety issues in production and handling (^{211}At is a gas). The first report on the use of $^{211}At^-$ as a substrate for NIS showed that although $^{211}At^-$ was transported by an I^- accumulating mechanism, probably involving NIS, neither ouabain nor perchlorate, both of which nearly abolish the transport of $^{125}I^-$, were able to fully suppress the basal-to-apical transfer of $^{211}At^-$.[43] It remains to be established whether this is an effect of active transport or is merely due to nonspecific adsorption of free $^{211}At^-$ to a "sticky" apical cell surface. Later reports on the use of $^{211}At^-$ for NIS-mediated therapy were more encouraging. Carlin and coworkers showed that [$^{211}At^-$]astatide uptake in the UVW human glioma cell line transfected to express NIS was NIS-dependent, with characteristics similar to $^{131}I^-$ uptake.[44]

Petrich and colleagues constructed a mammalian NIS expression vector and generated 6 stable NIS-expressing cancer cell lines, 3 derived from thyroid carcinoma, 2 from colon carcinoma, and 1 from glioblastoma.[45] Compared with the respective control cell lines, steady-state radionuclide uptake in NIS-expressing cell lines increased up to 350-fold for $^{123}I^-$, 340-fold for ^{99m}Tc-pertechnetate, and 60-fold for $^{211}At^-$. Cellular $^{211}At^-$ accumulation was dependent on extracellular Na^+ ions and displayed a similar sensitivity towards Na^+ perchlorate inhibition to that of both radioiodide and $^{99m}TcO_4^-$ uptake. Heterologous competition with unlabeled NaI decreased NIS-mediated $^{211}At^-$ uptake to the levels observed in control cells devoid of NIS. Following uptake, radioiodide and $^{211}At^-$ were rapidly released (apparent half-life 3 to 15 minutes) by the cells, as determined by wash out experiments. Data on scintigraphic tumor imaging in a xenograft nude mouse model of transplanted NIS-modified thyroid cells indicate that radionuclide uptake in NIS-expressing tumors was up to 70 times $^{123}I^-$, 25 times $^{99m}TcO_4^-$, and 10 times $^{211}At^-$ higher than in control tumors or normal tissues, except stomach (3 to 5 times) and thyroid gland (5 to 10 times). Compared with cell culture experiments, the effective half-life in vivo was highly prolonged (ie, 6.5 hours for $^{123}I^-$, 5.2 hours for $^{211}At^-$).

The remarkable progress made in the molecular characterization of NIS in the last few years has clearly created new opportunities for the development of diagnostic and therapeutic applications for NIS.[2,3,46-52] As discussed throughout this article, these applications are especially relevant in the realm of nuclear medicine, creating the prospect of using administered radioisotopes in a highly targeted and effective fashion to destroy a wide variety of cancer cells by internal, rather than external, radiation.

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