Review

BRJ
Biomedical Research Journal

Cancer gene therapy: Prospects of using human sodium iodide symporter gene in non-thyroidal cancer

Shruti Dutta and Abhijit De*

Molecular Functional Imaging lab, Advanced Centre for Training, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, India

Gene therapy is one of the promising therapeutic strategies evolved rapidly in the frontier of translational biology in cancer. To overcome the off target effect of conventional cancer therapies it is the most flourishing approach in present epoch. Various researches in this context are ongoing to eradicate devastating cancer cells with minimal or no side effects. Of the various gene therapy protocols developed, a set of genes called suicide genes, are being actively pursued as potential strategy. Briefly, this strategy involves tumor targeted delivery of a therapy/reporter gene to convert a systematically administered pro-drug into a cytotoxic drug which in turn induces tumor cell death. Additionally, advancement in small animal imaging modalities facilitates real-time monitoring of the delivered transgene by using appropriate imaging probe developed against the transgene. Non-invasive monitoring helps to realize precise transgene delivery and also aid to understand therapy response. In this background, we have reviewed potential suicide genes frequently explored for cancer treatment, which supports both diagnostic and therapeutic applications with special emphasis on sodium iodide symporter (NIS). Apart from its natural expression in thyroid, NIS protein expression has raised the possibility of using radioiodide therapy and diagnosis in few non-thyroidal cancers as well. In this review, we also covered various challenges to get NIS gene therapeutics from bench to bedside in various non-thyroidal cancers.

Gene Therapy for Cancer

With rapid advances in cellular and molecular understanding in the genome era, gene therapy holds great potential in treating various human diseases including cancer. The science behind gene therapy relies on introducing genes to cure or retard the progression of the disease. Theoretically, by introducing necessary modifications for the mutated part(s) of a gene or by replacing the defective gene as a whole, one can potentially cure or retard the severity of a disease caused by the effect of a single gene. However, in reality, cancer is mostly considered as a multi-gene disorder (LaDuca *et al*., 2014). Though many cancers have a genetic predisposition, a majority of them have

Key words: Sodium iodide symporter, Breast cancer, Histone deacetylase inhibitors, Radioiodine therapy, Molecular imaging.
***Corresponding Author:** Abhijit De, Molecular Functional Imaging lab, Advanced Centre for Training, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, India.
Email: ade@actrec.gov.in

acquired mutations and chromosomal abnormalities. As the disease progress, the cells become less differentiated and more heterogeneous with respect to the mutations they carry. The range of genes involved or the mutations they carry have grown into a long-winded task for gene therapy field to solve. The ability to image the location(s), magnitude, and real-time variation of therapeutic gene expression has become a key force in the rapid evolution of gene therapy. Another concern in gene therapy application is the need to achieve controlled and effective gene expression in the target cells, with minimal or no off-target effect in neighboring normal tissue locations. To address this issue, *ex vivo* strategies help to ensure that gene transfer is limited to cells of a particular organ. For example, gene transfer into bone marrow cells provides a means to introduce genes selectively into various types of blood cells, including hematopoietic stem cells (Pan, 2009). However, ability of direct gene transfer to the appropriate cells via systemic delivery of a vector is often complicated, but appreciably enhances gene therapy approaches.

Coming to gene therapy of cancer, diverse applications reported in literatures can be broadly categorized into five subgroups based on their mechanism of actions: (i) suicide gene therapy which uses introduction of drug-sensitive genes for selective induction of cellular sensitivity to a prodrug, (ii) protection of sensitive tissues like bone marrow from otherwise toxic doses of a cancer drug by use of multidrug resistance genes, (iii) replacement of lost or loss-of-function tumor suppressor genes, (iv) compensating down-regulated oncogene or gain-of function oncogenic mutation, and (v) insertion of a cytokine gene into tumor cells *ex vivo*. In this review, however, we want to focus on the first approach, where the gene or transgene signature can be utilized for killing the same cells where it is being expressed. We will continue our discussion analyzing gene therapy applications using sodium iodide symporter (NIS) in various non-thyroidal cancers.

Suicide Gene Therapy

Eventually the need of a cancer therapy is for complete remission of the cancer cells causing minimal damage to the surrounding normal tissues. In this regard the most promising approach is the targeted suicide gene therapy. With recent

advances in vector design, improvements in transgene (a new or altered gene that is being introduced) and prodrug activation strategies, suicide gene therapy is being applied to a wide variety of cancers (Zarogoulidis *et al*., 2013). Till date various prokaryotic or eukaryotic genes have been tested as suicide gene therapy candidate, several of them also support diagnostic imaging to identify *in vivo* localization of the gene in action. For a particular therapeutic gene, diagnosis was done mostly by using gamma-ray emitting radionuclide probes while beta-ray emitting radionuclide probes are used for cancer cell diminution. In order to understand the consequences of the delivered transgene, non-invasive and real-time monitoring by using appropriate imaging modality is crucial. In this context reporter gene that supports radionuclide-based imaging approach gains attention as these imaging procedures are clinically relevant. With improved optical imaging instrumentations, the radionuclide based imaging applications has expanded scope now. Based on the phenomenon known as Cerenkov luminescence, emits visible luminescence photons when the charged radioactive particles travel through a dielectric media (such as tissue) at a speed higher than the speed of light, can be captured in real time to understand the tissue bio-distribution of radiotracers (reviewed in Thorek *et al*., 2012, Tanha *et al*., 2015). There are at least three different types of interactions between the reporter proteins with their probes, which include an enzyme-based (e.g. Thymidine kinase and Cytosine deaminase), receptor-based (e.g. Somatostatin receptor) and transporter-based (e.g. NIS), used frequently for cancer gene therapy. We will now discuss ongoing research efforts utilizing these genes.

Herpes Simplex Virus type 1 Thymidine kinase (HSV-1 TK)

Among different suicide genes HSV-1 TK is the most frequently studied classical suicide gene target which converts non-toxic prodrug into a toxic drug. This viral TK phosphorylates various nucleoside analogues like aciclovir, ganciclovir, penciclovir much more efficiently than its mammalian homolog. Thus, mechanistically these cell-permeable substrates first get monophosphorylated by HSV-1 TK and subsequently phosphorylations are carried out by host kinase to generate the triphosphate form, which (deoxy-thymidine triphosphate) is basically a purine analog that inhibits DNA polymerase and therefore creates

toxicity to cause cell death eventually. Being highly proliferative in nature, cancer cells actively synthesize DNA, so the purine analog competes with guanosine triphosphates (GTPs) and get incorporated into the nascent DNA chain. As a result the nuclear and mitochondrial DNA synthesis terminates and cells are forced towards apoptosis. However, in transgenic mice the use of HSV-1 TK for tissue specific sensitization through ganciclovir treatment showed limitations. Due to high nuclear localization, the enzyme creates spermatozoal toxicity which in turn renders the male transgenic mice sterile (Cohen *et al*., 1998). In another study investigators have revealed that due to the presence of putative cryptic testis-specific promoter within the coding sequence, the HSV-1 TK gene exhibited such outcome (Salomon *et al*., 1995). Therefore to address this issue various studies have been carried out by generating different mutated version of HSV-1 TK with improved enzymatic activity and varying nuclear clearance (Ponomarev *et al*., 2003). Among those HSV-1 sr39TK (also termed as HSV-1 tTK) was the most successful mutant (Black *et al*., 2001) used recurrently for radionuclide imaging as well as suicide gene therapy purpose. Imaging of HSV-1 tTK using ^{18}F-9-(4-[18F] fluoro-3-hydroxymethylbutyl) guanine (^{18}F-FHBG) and ^{18}F- or ^{124}I-2′-deoxy-2′-fluoro-5-iodo-1-[β]-D-arabino-furanosyluracil (^{18}F-FIAU and ^{124}I-FIAU) positron emission tomography (PET) turned it into a imaging reporter for clinical use. Later successful combining strategies by fusing HSV-1 sr39TK to other reporters made it suitable for multimode imaging (De *et al*., 2003; Ray *et al*., 2003; Ray *et al*., 2004; Ruggiero *et al*., 2010; Serganova *et al*., 2008). In another study, a triple fusion gene construct using NIS, HSV1-sr39tk, and EGFP was developed and demonstrated its use as a suicide therapy agent in hepatocellular carcinoma (Lee *et al*., 2010). However majority of such fusion gene using HSV-1 TK are used as imaging reporter rather than suicide therapy purpose. Another therapeutic study noteworthy here is the first-in-man use of engineered T-cells with HSV-1 TK-truncated CD34 fusion. This work highlights the suitability of tCD34 as a GMP compliant selection marker and demonstrates the feasibility, safety and immunological potential of HSVTK-tCD34 suicide gene modified donor T cells (Zhan *et al*., 2013).

Cytosine deaminase (CD)

Cytosine deaminase protein is mainly synthesized in some bacteria and fungi, which deaminates cytosine to uracil. CD can also converts a non-toxic compound 5-fluorocytosine (5-FC) into the toxic compound 5-fluorouracil (5-FU) (Ramnaraine *et al*., 2003). It exerts toxic effect by replacing for uracil in cellular RNA and therefore interferes with DNA and protein synthesis. Basically the absence of CD in mammalian tissues allows its use as a drug for suicide gene therapy for various cancer treatments. In an *in vitro* study genetically engineered stem cells to produce CD converts non-toxic 5-FC to a cytotoxic agent 5-FU and after migrating towards tumor site exhibited reduction in tumor growth (Kim *et al*., 2010). Deamination of 5-FC prodrug produces two toxic metabolites such as 5-fluorodeoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FURTP). FdUMP being a potent inhibitor of thymidylate synthetase, an enzyme required for DNA synthesis, inhibits DNA synthesis and endorses apoptosis in tumor cells (Chen *et al*., 2007; Myers, 1981). In the first attempt suicide gene therapy using CD was demonstrated significant tumor reduction in rat glioma cells using 5-FC (Nishiyama *et al*., 1985). Several other studies have also demonstrated positive effect of CD/5-FC as an effective anti-tumorigenic system for therapy in different other cancers (Li *et al*., 2003; Lv *et al*., 2009; Yang *et al*., 2015). Improved tumor regression was obtained when radiation was combined with adenoviral mediated delivery of a mutated CD gene (Ad bCD-D314A) in pancreatic cancer (Kaliberova *et al*., 2008).

Nitro-reductase (NTR)

Another promising, but relatively less used, prodrug activation enzyme used in cancer therapy is nitro-reductase (NTR). NTR is a flavoprotein synthesized by *Escherichia coli*. One of its substrates is CB1954 (5-[aziridin-1-yl]-2, 4-dinitro-benzamide), which reacts with cellular thioesters and get converted into a potent DNA cross-linking agent by NTR resulting in inhibition of DNA synthesis. Therefore viral mediated delivery of NTR in tumor cells get sensitized upon administration of CB1954, demonstrated the basis of cancer gene therapy (Searle *et al*., 2004). An *in vitro* study showed that upon CD1954 treatment, NTR expressing clones (retro virus mediated delivery) of pancreatic and colorectal cancer cell lines

became 500 and 50 fold more sensitive than the parental cell line respectively (Green *et al*., 1997). In preclinical tumor xenograft model, tumor regression using NTR/CD1954 was also reported (McNeish *et al*., 1998; Weedon *et al*., 2000).

Somatostatin receptor (SSTR)

Somatostatin (SST) is a peptide hormone involved in various biological processes in normal human tissues. When SST interacts with SSTRs, it exhibits strong anti-proliferative effect in normal and tumor cells. SSTRs are consisting of G-protein coupled receptor subtypes (1-5), differentially expressed in various tumor types. Among these subtypes SSTR1 and SSTR2 has been reported to have pivotal role in anti-proliferative effect. However, SSTR2 is majorly studied in gene therapy for cancer treatment (Schaer *et al*., 1997). In most of the prostate cancer cases SSTR2 expression is found to be inactivated, so when the full length cDNA of SSTR2 was introduced using non-viral gene delivery system in the PC-3 cells, marked anti-angiogenic effect was reported (Kumar *et al.*, 2004). An *in vitro* study in pancreatic carcinoma also showed significant inhibition in cell proliferation upon delivery of an adenoviral mediated MUC1-promoter expressing SSTR2, although there was no AdMUC1-SSTR2-induced apoptosis (Chen *et al.*, 2005). *In vivo* study also showed impairment of tumor progression upon delivery of SSTR2 gene in pancreatic cancer (Carrere *et al.*, 2005). Interestingly neuro-endocrine tumors (NETs) have natural over expression of SSTRs, so detection and treatment of NETs become easier by using SSTRs. ^{68}Gallium (^{68}Ga) labeled somatostatin analogue such as 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetra-acetic acid (DOTA) was used to image NETs through PET/CT which was found to be more efficient than the conventional SSTR scintigraphy (Gabriel *et al.*, 2007; Sollini *et al.*, 2014). Moreover, other somatostatin analogues like ^{177}lutetium (^{177}Lu) or ^{90}yttrium (^{99}Y) are also found to have potential use in therapy for NET patients (Kam *et al*., 2012; Sowa-Staszczak *et al*., 2011).

Sodium iodide symporter (NIS)

Human NIS expression in various cancers like thyroidal and several non-thyroidal malignancies allow its worldwide use for theranostic application. Being a member of the solute carrier transporter (SLC5A5),

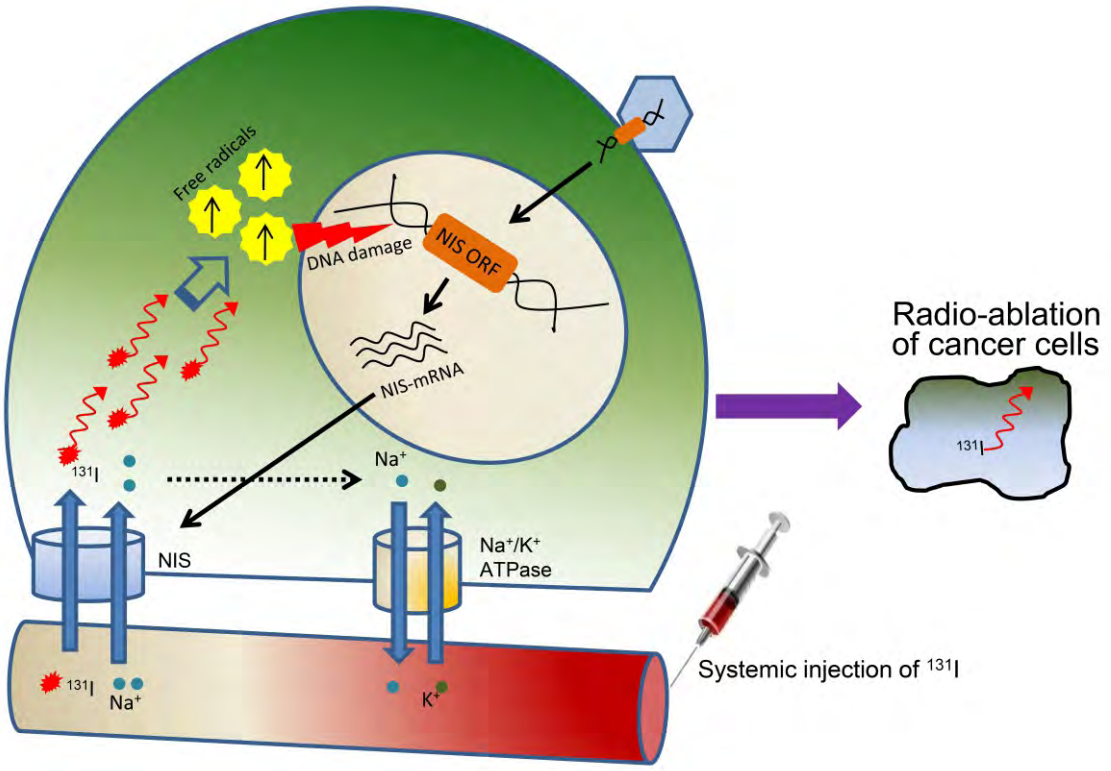

Figure 1: Mechanistic representation of NIS mediated gene therapy using I^{131} administration. Being a Na^+/I^- pump protein, NIS imports 2 Na^+ and one I^- across the cell membrane where it expresses. Thus after achieving functional NIS expression administered I^{131} permits selective ablation of neoplastic cells.

NIS is an intrinsic plasma membrane glycoprotein that mediates active iodide transport in thyroid follicular cells. NIS mediated iodide transport is also seen in extra-thyroidal tissues such as salivary gland, gastric mucosa and lactating mammary tissue. The natural function of this protein allows inward iodine transport to the cells providing the opportunity to use it for radioiodine mediated diagnosis and therapy (Fig. 1). Among various radionuclides ^{123}I, ^{125}I, $^{99m}TcO4$ are mainly used for SPECT (single photon emission computed tomography) and ^{124}I for PET based diagnostic imaging application while β-emitting ^{131}I is used for therapeutic application. However, measurement of therapeutic efficacy through imaging is often complicated as that will require delivery of two different radiotracers. Therefore, use of a surrogate marker (e.g. ^{18}F-FDG PET scan) or an independent reporter based imaging (e.g. bioluminescence/fluorescence imaging) to monitor therapy effect can help circumventing the issue (Fig. 2A). For experimental and preclinical imaging applications, of course Cerenkov

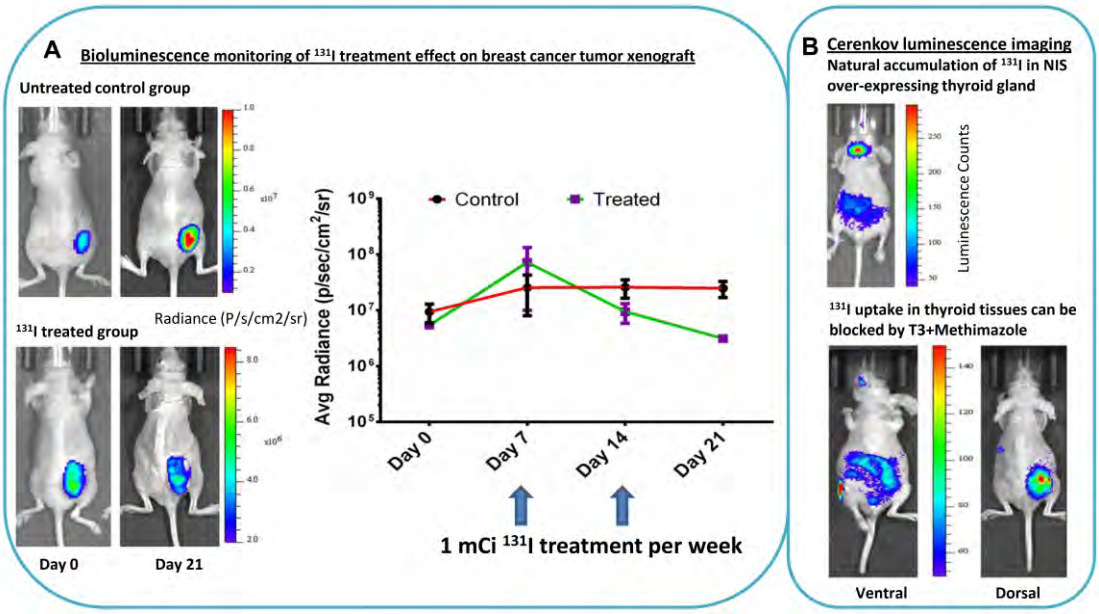

Figure 2: Optical luminescence imaging of breast cancer xenograft in mice undergoing ^{131}I treatment. A. Bioluminescence imaging to evaluate ^{131}I therapy in mice. These sets of mice were implanted with Zr75-1 breast cancer cells co-expressing human NIS and Firefly luciferase reporter gene. After a good size of tumor develops, mice in the treatment group received weekly dose of 1 milliCurie/mouse ^{131}I on day 7 and 14 as indicated and monitored for attenuation in luciferase signal by injecting D-luciferin substrate. In comparison to the untreated control group, treated group shows significant diminution in tumor luciferase signal indicating NIS expressing tumor cell death. B. ^{131}I being -emitter generates Cerenkov luminescence signal. This signal can be monitored using optical CCD camera. Representative mouse image on top shows natural accumulation of ^{131}I in thyroid gland 24 hours after 1 milliCurie radioiodine was injected intra-peritoneal. Bottom image shows ventral and dorsal view of a tumor bearing mouse in which breast cancer xenograft was placed dorsally on the right flank. This mouse received 14 days pre-treatment with triiodothyroxin (T3) and methimazole (an iodine organification inhibitor) daily, showing possibility of thyroid blocking without affecting the radioiodine uptake in breast tumor xenograft.

luminescence imaging (Thorek *et al.*, 2012; Xu *et al.*, 2012) from most of these particulate emitters (except for ^{99m}TcO4) supported by optical imaging modalities can be exploited (Fig. 2B). The ability of various radioisotopes producing Cerenkov radiation definitely add advantages such as low scan time requirement providing higher throughput and quantitative measurement of radioisotope distribution in the body, however the modality also suffers from issues such as lack of absolute quantitative ability and significant signal attenuation with greater tissue depths.

Gene therapy applications using NIS can be broadly categorized into two streams, transgene-mediated and endogenous and we will now discuss various cancer application aspects in detail in the following sections.

Sodium Iodide Symporter as a Transgene Target for Cancer Treatment

Since, human NIS protein functions in a

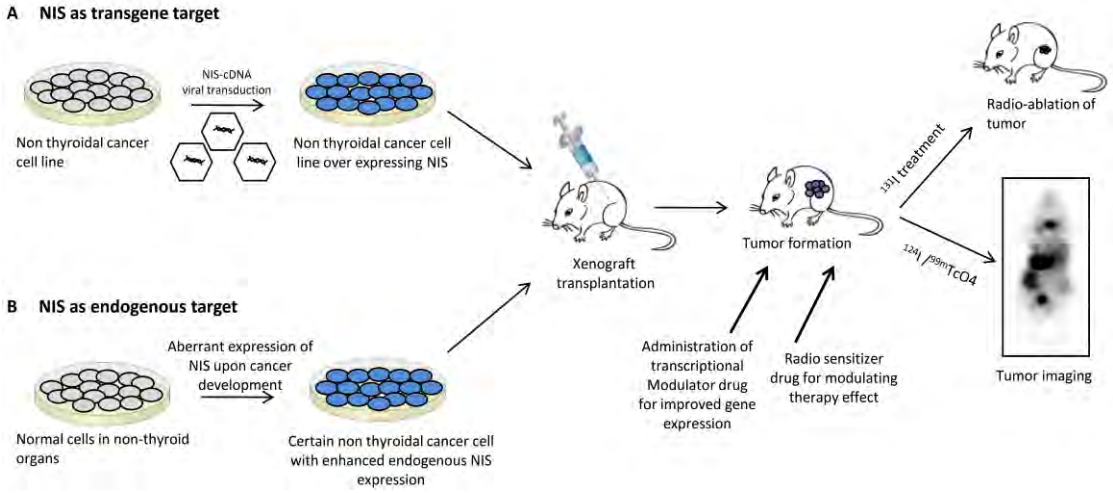

Figure 3: Schematic explaining various preclinical experimental set up for testing NIS gene mediated diagnosis and therapy in non-thyroidal cancers. A. NIS as transgene target is generally achieved by viral transduction methods in cancer cells. Such NIS over-expressing cells can be used for xenograft transplantation. Tumor formation can be visualized through appropriate imaging modality by administering ^{124}I / $^{99m}TcO4$. Treatment with ^{131}I leads to tumor ablation. B. NIS as endogenous target is often recorded in certain cancer types. Various pharmacological modulators can be tested to improve endogenous NIS expression and/or function in these cancer cells *in vitro* or *in vivo* tumor xenograft. Radio-sensitization of tumor could further improve treatment efficacy though ^{131}I treatment.

very limited number of tissues in human body (primarily in thyroid, salivary gland, gastric mucosa and lactating breast), it is an attractive target to treat cancer in tissue types where NIS has no/aberrant expression. Among various non-thyroidal cancers NIS (isolated from both rat and human) transgene mediated targeted radioiodine therapy was studied primarily in prostate, colon, ovarian and breast cancers (BCs). Adenovirus and retrovirus mediated NIS gene transfer has been attempted specifically to these tumor cells and in some cases there *in vivo* efficacy was also tested (Fig. 3A). NIS gene delivery in prostate cancer has been thoroughly explored since last decades. The first attempt of an *in vivo* adenovirus mediated hNIS (human NIS) delivery was done by Spitzweg et al. in non-thyroidal tumor xenograft. Significant NIS gene expression in prostate cancer xenograft was obtained followed by average reduction of tumor volume upon treatment with therapeutic ^{131}I (Spitzweg *et al.*, 2001). Later adenovirus mediated delivery of rat NIS (Ad-rNIS) was also reported in human prostate cancer tumor xenograft. The study also showed inhibition of tumor growth after intratumoral injection of Ad-rNIS followed by ^{131}I treatment (Spitzweg *et al.*, 2001). Therefore it is quite evident that rNIS has an equal potential compared to hNIS in suicide gene therapy for cancer

treatment. Further investigation has revealed that virus mediated NIS gene expression could be up-regulated through external beam radiation and DNA damage repair inhibitors (Hingorani *et al.*, 2008a; Hingorani *et al.*, 2008b). Additionally administration of radiosensitizing drugs further enhance therapeutic potential of NIS mediated ^{131}I in colorectal and head and neck cancer (Hingorani *et al.*, 2010). Baculovirus mediated NIS gene delivery in colon cancer cell line also showed efficient cell death through ^{131}I therapy (Yin *et al.*, 2010). In an *in vitro* study using ovarian cancer cell transduced with Ad/CMV/NIS and Ad/MUC1/NIS, 12- and 5-fold higher iodide uptake was reported compared to the parental cell line respectively. The same group has also shown reduction of ovarian tumor xenograft following ^{131}I treatment upon CMV/NIS infection of the tumors (Dwyer *et al*., 2006).

On the other hand, in BC, aberrant over-expression of NIS is already reported; however the level of expression is often not sufficient for any therapeutic application. So, to achieve effective radio-iodine therapy, investigators adopted strategy to selectively transfer NIS gene in breast cancer cell types. In one of such studies engineered MCF7 cell line over expressing NIS and firefly luciferase was used to create mice xenograft and tested the effect of ^{131}I through bioluminescent imaging. The pattern of tumor regression upon ^{131}I administration in NIS expressing BC xenograft was also shown (Ghosh *et al*., 2006). Another study has generated a conditional adenovirus vector containing MUC1 promoter driven E1a gene and a transcriptional cassette RSV promoter-hNIS in the E3 region that can replicate only in the MUC1 positive cells. Therefore, the engineered virus particles were transduced into MUC1 positive T47D breast cancer cell line showing improved radioiodine uptake (Trujillo *et al.*, 2009). Interestingly, virus mediated NIS transgene expression was shown to be further induced by treating cancer cells with retinoic acid (RA) which also showed subsequent enhancement in radioiodine uptake in MCF7 cell lines (Lim *et al.*, 2007). Further, non-replicative adenovirus was used for targeted NIS gene delivery by using promoters of human telomerase subunits RNA (hTR) and human telomerase reverse transcriptase (hTERT), which are active only in cancer cells (Riesco-Eizaguirre *et al.*, 2011).

Overcoming challenges of NIS gene delivery

Although viral mediated NIS gene delivery is mostly attempted but this approach poses some risks for human application. The virus particles having their natural tropism towards certain cell types may have a natural tendency to infect and integrate and/ or re-combine within the genome. For example, in an initial clinical trial for NIS gene therapy in X linked severe combined immuno-deficiency, an incidence of leukemia was reported due to unexpected integration of the viral DNA in the host genome (Kogai *et al*., 2012). Therefore to avoid such unwanted DNA integration, all experimental NIS gene therapies are conducted with replication deficient viral vectors (Boland *et al*., 2000; Hutzen *et al*., 2012; Kim *et al*., 2007). However, immunogenic reactions by the host, mutagenic integration (retroviral and lentiviral vectors), inflammatory toxicity (adenoviral vectors), and large scale production of the viral particles (adeno-associated vectors) are major limitations for such applications (Duarte *et al.*, 2012; Witlox *et al.*, 2007). So, current attempts are oriented towards non-viral vectors as vehicles for gene/drug delivery. Cationic lipids, polymers, peptides and nano-particles have commonly been used for DNA delivery into the cells in this regard (Chen *et al*., 2015; Duarte *et al*., 2012; Fan *et al*., 2015;). Unlike viral vectors, targeting of non-viral vectors is a major concern and that could be improved by conjugating non-viral vectors with ligands which bind to specific receptors or antigens expressed on the cancer cells (Ogris *et al*., 2002).

Sodium Iodide Symporter as an Endogenous Target for Cancer Therapy

Apart from introducing NIS gene in various cancer cells, ongoing active research has also exploited therapeutic potential of the gene where a specific cancer type is associated with aberrant over-expression of this gene. In such cases, since NIS protein is already present in the tumor cells, the burden of designing a vector for delivering the gene in the target cell is eliminated and thus can be spontaneously utilized for therapeutic intervention using radioiodine. Here, we have discussed various studies which have attempted ways to improve endogenous NIS expression and function for optimal therapeutic benefit (Fig. 3B). NIS is a true theranostic molecule which supports non-

invasive *in vivo* imaging using different form of radioactive iodine (PET, SPECT radiotracers) to judge sufficient iodine accumulation inside the tumor, which in turn indicates therapeutic success. Endogenous NIS gene therapy applications are primarily aimed at breast cancer. Apart from BC, modulation strategy to alter NIS aberrant expression was so far known from hepatocellular and testicular cancers (Guerrieri *et al*., 2013; Maggisano *et al*., 2014). In rat Leydig testicular carcinoma cells (LC540) treatment of HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and valproic acid (VPA) in combination showed enhanced NIS expression both in transcript and protein level with subsequent improvement of radioiodine uptake (Maggisano *et al*., 2014). Evaluation was also done in liver cancer cells and correlated with p53 family member proteins showing increased NIS expression (Guerrieri *et al*., 2013).

In the context of BC, of the various known subtypes, more than 80% hormone receptor positive cases (estrogen and progesterone receptor) were reported to have natural expression of NIS protein, while around 65% triple negative breast cancer (TNBC) cases showed positive expression (Chatterjee *et al*., 2013; Tazebay *et al*., 2000; Wapnir *et al*., 2003). Taking these results forward, clinical studies have been carried out to verify the translational potential of NIS in malignant BC patients. Moon *et al.* (2001) reported significant $^{99m}TcO_4^-$ uptake in 4 out of 25 breast cancer patients by scintigraphic scanning method. Another important study by Wapnir *et al.* detected radioiodine uptake in metastatic BC by scintigraphic analysis using radioiodides (^{123}I and ^{131}I) or $^{99m}TcO_4$. However, surprisingly iodine uptake was noted in only 25% (2 out of 8) of NIS positive metastatic breast tissues (Renier *et al*., 2009; Wapnir *et al*., 2004). Therefore, current focus on achieving effective NIS gene therapy is to find potent modulators that could improve endogenous NIS expression and/ or function.

Overcoming challenges of NIS gene therapy

As mentioned above, exploiting endogenous NIS as a therapeutic target is limited by its ability to pump sufficient iodine inside cells, especially in non-thyroidal cancer tissues such as breast. This discordant is primarily due to lack of NIS expression on plasma membrane,

which is critical for its iodine transporter function delivering iodine inside the cell. In this regard, epidermal growth factor (EGF) was identified to localized NIS better on the plasma membrane in non-thyroidal cancers, while reverse localization was also obtained by treatment with a MEK-1 inhibitor, suggesting involvement of MEK-ERK signaling pathway in NIS localization (). Moreover, as far as improvement of NIS endogenous expression is concerned, trans-retinoic acid (tRA) was frequently studied as the major inductor in BC. Treatment with tRA showed significant increase in ^{131}I mediated radioablation in MCF7 cell lines (Kogai *et al*., 2012). Besides, *in vivo* study using MCF7 xenograft mouse model showed enhanced radioiodine uptake sufficient for effective cell killing (Kogai *et al*., 2004). But due to short biological half-life, the effect of tRA exists in the system only for a limited time period and therefore requires frequent use during therapeutic intervention. However, frequent use of tRA caused cardio-respiratory distress syndrome in patients of acute promylocytic leukemia (Warrell, 1993). 13-cis RA can be used as a prodrug which finally gets converted into tRA inside the target cells. Experimental study has shown that although it induces NIS expression, the level of expression is much lower than tRA administration (Kogai *et al*., 2005). RAs operate by binding through two families of nuclear receptors, retinoic acid receptors (RARs), which are activated by both all-trans RA and 9-cis-RA, and retinoid X receptors (RXRs), which are activated by 9-cis-RA only. Upon binding of RAs, receptors get activated and bind to RA responsive elements in the promoter regions of target genes and work as ligand-dependent transcription factors (Alotaibi *et al*., 2010).

Primarily, NIS activation takes place by binding of RA to RARα/RXRβ receptor hetero-dimer followed by activation of the phosphoinositide 3-kinase (PI3K) pathway and the p38βMAPK pathway (Kogai *et al*., 2012). By administering PI3K inhibitor or by knock down of p85α (a regulatory subunit of PI3K) showed decreased RA induced NIS expression in MCF7 cells. Decrease in iodide uptake was also reported upon inhibition of AKT pathway in MCF7 cell lines (Kogai *et al*., 2012) suggesting involvement of PI3K/AKT pathway in NIS induction. Further, combination of tRA with several pharmacological compounds found to be

more effective in NIS gene induction in non-thyroidal cancers. Such combination therapies used drugs like hydrocortisone, dexamethasone (Dex) (Dohan *et al.*, 2006; Kogai *et al.*, 2005), troglitazone (a peroxisome proliferator–activated receptor γ, PPARγ, agonist) (Tanosaki *et al.*, 2003; Wei *et al.*, 2009), various histone deacetylase (HDAC) inhibitors (tricostatin A and sodium butyrate), and carbamazepine, an agonist of pregnane X receptor (Unterholzner *et al.*, 2006; Willhauck *et al.*, 2011). Some other compounds like prolactin, insulin, and insulin growth factor (IGF)-I and II were also used to stimulate NIS mRNA expression in BC cell line (Arturi *et al.*, 2005).

Moreover, the efficiency of radioiodine therapy in various cancers not only depends on NIS protein expression and its membrane localization, the factor that can't be ignored is biological half-life and retention potential of radioiodine in the tumor bed. In thyroid gland, due to iodine organification the biological half-life of iodine is sufficiently long, making radioiodine therapeutics effective for thyroid cancer treatment (Shimura *et al.*, 1997). However in lactating mammary gland approximately 20% of the iodine was trapped due to iodine oxidation by lactoperoxidase (LPO) expressed in alveolar cells followed by binding of iodine to various milk proteins (Etling *et al.*, 1984; Strum *et al.*, 1983). But this may be insufficient for radioiodine therapy in BC. So, studies are underway to improve iodine retention time in breast cancer cells for getting ^{131}I therapy effective. Combination treatment with all-trans RA and Dex has shown modest improvement in iodine retention in MCF7 cells. However, the exact mechanism is not known (Unterholzner *et al.*, 2006).

Furthermore, as NIS is highly expressed in normal thyroid tissues, while treating non-thyroidal cancers safeguarding thyrocytes can also be a major issue. However, this issue has already been addressed by selective down-regulation of NIS expression in thyrocytes by administering T3 and methimazole in combination (Wapnir *et al.*, 2004). Apart from thyroid and lactating mammary gland NIS is also expressed in several other normal tissues such as salivary gland, intestinal epithelium, lacrimal gland, stomach lining etc. that become barrier for NIS based imaging and treatment. Uptake in these normal tissues not only reduces therapeutic efficiency but

subsequently radioiodine damages of normal organ function as well.

Clinical Experiences with NIS

NIS based diagnosis and therapy is well known in thyroid clinics for several decades to identify and treat various thyroid diseases including cancer. In a recent case study after complete thyroidectomy in differentiated thyroid cancer patient, whole-body scans based on diagnostic or therapeutic doses of ^{131}I can visualize various distant metastatic lesions. Nonetheless, extreme precautionary measure is required while analyzing the data because several false positive signals were also obtained (Ahn *et al.*, 2011). As per the NIH database (www.clinicaltrials.gov), at present there is no ongoing or completed clinical trials on NIS based therapy in non-thyroidal cancers. The reasons are indeed due to several such issues that are discussed above. So, investigators are actively investigating effective methods of modulation to enhance NIS expression and localization on plasma membrane to achieve the optimal efficacy. However, recently few clinical trials in prostate and ovarian cancer are attempted for NIS gene therapy. In a phase I clinical trial, prostate cancer patients received intra-prostatic injection of Ad5-yCD/mutTK(sr39)rep-hNIS followed by measuring hNIS expression over time through SPECT imaging after adenovirus injection. Positive hNIS expression was obtained in the patient's prostate gland suggesting non-invasive imaging of NIS gene is achievable and safe for humans (Barton *et al*., 2008; 2011). Moreover this study also proved the application of ^{131}I in human for localized prostate cancer treatment. Recently a clinical study in ovarian cancer also showed promising observation upon intra-peritoneal administration of engineered measles virus to express NIS. NIS expression was confirmed in patient's tumor through ^{123}I uptake using SPECT/CT scan (Galanis *et al.*, 2015). Further clinical evaluations are expected in the coming years to achieve successful NIS gene therapy application in non-thyroidal cancers.

CONCLUSIONS

Gene therapy in cancer has high potential because of recent advancement in genetic engineering in cellular and molecular level. In this arena, NIS gene gains importance in cancer for gene therapy because of its property to serve both

diagnostic imaging and therapeutics. Moreover to improve therapeutic effectiveness, NIS gene therapy is rapidly evolving in various non-thyroidal cancers particularly in BC because of lack of appropriate therapeutic options in hormone receptor negative patients. Although NIS gene therapy is already on track for various thyroid cancers, but for non-thyroidal cancers there are still various logistics that need to be addressed before successful clinical translation. Major concerns which need to be taken care are the expression level, protein localization on cell membrane and lower retention time of radioiodine in cancer tissues. So, optimal strategies are yet to be developed to improve radioiodine uptake and retention by modulating NIS gene expression in non-thyroidal cancers. Expectedly, the progression in recent basic research unraveling NIS biology in the field of gene therapy would develop right strategies of treatment to care devastating cancer.

ACKNOWLEDGMENTS

Funding support from TMC-Woman Cancer Initiative funding (#82) and ICMR, New Delhi (Ref. No. 5/13/25/10/NCD-III) to AD and DST-WOSA award to SD is gratefully acknowledged. For some parts of the figure materials, we also acknowledge the work done by Sushmita Chatterjee in the lab.

CONFLICT OF INTEREST

The authors claim no conflict of interest.

REFERENCES

Ahn BC, Lee SW, Lee J, Kim C. Pulmonary aspergilloma mimicking metastasis from papillary thyroid cancer. *Thyroid* 2011;21: 555–558.

Alotaibi H, Yaman E, Salvatore D, Di Dato V, Telkoparan P, Di Lauro R, *et al*. Intronic elements in the Na+/I- symporter gene (NIS) interact with retinoic acid receptors and mediate initiation of transcription. *Nucleic Acids Res* 2010;38:3172–3185.

Arturi F, Ferretti E, Presta I, Mattei T, Scipioni A, Scarpelli D, *et al*. Regulation of iodide uptake and sodium/iodide symporter expression in the mcf-7 human breast cancer cell line. *J Clin Endocrinol Metab* 2005;90:2321–2326.

Barton KN, Stricker H, Brown SL, Elshaikh M, Aref I, Lu M, *et al*., Phase I study of noninvasive imaging of adenovirus-mediated gene expression in the human prostate. *Mol Ther* 2008;16:1761–1769.

Barton KN, Stricker H, Elshaikh MA, Pegg J, Cheng J, Zhang Y, *et al*. Feasibility of adenovirus-mediated hNIS gene transfer and 131I radioiodine therapy as a definitive

treatment for localized prostate cancer. *Mol Ther* 2011;19:1353–1359.

Black ME, Kokoris MS, Sabo P. Herpes simplex virus-1 thymidine kinase mutants created by semi-random sequence mutagenesis improve prodrug-mediated tumor cell killing. *Cancer Res* 2001;61:3022–3026.

Boland A, Ricard M, Opolon P, Bidart JM, Yeh P, Filetti S, *et al*. Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for a targeted radiotherapy. *Cancer Res* 2000;60:3484–3492.

Carrere N, Vernejoul F, Souque A, Asnacios A, Vaysse N, Pradayrol L, *et al*. Characterization of the bystander effect of somatostatin receptor sst2 after in vivo gene transfer into human pancreatic cancer cells. *Hum Gene Ther* 2005; 16:1175–1193.

Chatterjee S, Malhotra R, Varghese F, Bukhari AB, Patil A, Budrukkar A, *et al*. Quantitative immunohistochemical analysis reveals association between sodium iodide symporter and estrogen receptor expression in breast cancer. *PLoS One* 2013;8:e54055.

Chen JK, Hu LJ, Wang D, Lamborn KR, Deen DF. Cytosine deaminase/5-fluorocytosine exposure induces bystander and radiosensitization effects in hypoxic glioblastoma cells in vitro. *Int J Radiat Oncol Biol Phys* 2007;67: 1538–1547.

Chen L, Liu Q, Qin R, Le H, Xia R, Li W, *et al*. Amplification and functional characterization of MUC1 promoter and gene-virotherapy via a targeting adenoviral vector expressing hSSTR2 gene in MUC1-positive Panc-1 pancreatic cancer cells in vitro. *Int J Mol Med* 2005;15: 617–626.

Chen Y, Nan J, Lu Y, Wang C, Chu F, Gu Z. Hybrid Fe3O4-Poly(acrylic acid) Nanogels for Theranostic Cancer Treatment. *J Biomed Nanotechnol* 2015;11:771–779.

Cohen JL, Boyer O, Salomon B, Onclerco R, Depetris D, Lejeune L, *et al*. Fertile homozygous transgenic mice expressing a functional truncated herpes simplex thymidine kinase delta TK gene. *Transgenic Res* 1998;7: 321–330.

De A, Lewis XZ, Gambhir SS. Noninvasive imaging of lentiviral-mediated reporter gene expression in living mice. *Mol Ther* 2003;7: 681–691.

Dohan O, De la Vieja A, Carrasco N. Hydrocortisone and purinergic signaling stimulate sodium/iodide symporter (NIS)-mediated iodide transport in breast cancer cells. *Mol Endocrinol* 2006;20:1121-37.

Duarte S, Carle G, Faneca H, de Lima MC, Pierrefite-Carle V. Suicide gene therapy in cancer: where do we stand now? *Cancer Lett* 2012;324:160–170.

Dwyer RM, Bergert ER, O'Connor MK, Gendler SJ, Morris JC, Sodium iodide symporter-mediated radioiodide imaging and therapy of ovarian tumor xenografts in mice. *Gene Ther* 2006;13:60–66.

Etling N, Gehin-Fouque F. Iodinated compounds and thyroxine binding to albumin in human breast milk. *Pediatr Res* 1984;18:901–903.

Fan Y, Moon JJ. Nanoparticle drug delivery systems designed to improve cancer vaccines and immunotherapy. *Vaccines (Basel)* 2015;3: 662–685.

Gabriel M, Decristoforo C, Kendler D, Dobrozemsky G, Heute D, Uprimny C, *et al*.

68Ga-DOTA-Tyr3-octreotide PET in neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and CT. *J Nucl Med* 2007;48:508–518.

Galanis E, Atherton PJ, Maurer MJ, Knutson KL, Dowdy SC, Cliby WA, *et al*. Oncolytic measles virus expressing the sodium iodide symporter to treat drug-resistant ovarian cancer. *Cancer Res* 2015;75:22–30.

Ghosh M, Gambhir SS, De A, Nowels K, Goris M, Wapnir I. Bioluminescent monitoring of NIS-mediated (131)I ablative effects in MCF-7 xenografts. *Mol Imaging* 2006;5:76–84.

Green NK, Youngs DJ, Neoptolemos JP, Friedlos F, Knox RJ, Springer CJ, *et al*. Sensitization of colorectal and pancreatic cancer cell lines to the prodrug 5-(aziridin-1-yl)-2,4-dinitrobenz-amide (CB1954) by retroviral transduction and expression of the E. coli nitroreductase gene. *Cancer Gene Ther* 1997;4:229–238.

Guerrieri F, Piconese S, Lacoste C, Schinzari V, Testoni B, Valogne Y, *et al*. The sodium/iodide symporter NIS is a transcriptional target of the p53-family members in liver cancer cells. *Cell Death Dis* 2013;4:e807.

Hingorani M, White CL, Merron A, Peerlinck I, Gore ME, Slade A, *et al*. Inhibition of repair of radiation-induced DNA damage enhances gene expression from replication-defective adenoviral vectors. *Cancer Res* 2008a;68: 9771–9778.

Hingorani M, White CL, Zaidi S, Merron A, Peerlinck I, Gore ME, *et al*., Radiation-mediated up-regulation of gene expression from replication-defective adenoviral vectors: implications for sodium iodide symporter gene therapy. *Clin Cancer Res* 2008b;14:4915–4924.

Hingorani M, White CL, Zaidi S, Pandha HS, Melcher AA, Bhide SA, *et al*. Therapeutic effect of sodium iodide symporter gene therapy combined with external beam radiotherapy and targeted drugs that inhibit DNA repair. *Mol Ther* 2010;18:1599–1605.

Hutzen B, Pierson CR, Russell SJ, Galanis E, Raffel C, Studebaker AW. Treatment of medulloblastoma using an oncolytic measles virus encoding the thyroidal sodium iodide symporter shows enhanced efficacy with radioiodine. *BMC Cancer* 2012;12:508.

Jung KH, Paik JY, Ko BH, Lee KH. Mitogen-activated protein kinase signaling enhances sodium iodide symporter function and efficacy of radioiodide therapy in nonthyroidal cancer cells. *J Nucl Med* 2008; 49:1966–1972.

Kaliberova LN, Della Manna DL, Krendelchtchikova V, Black ME, Buchsbaum DJ, Kaliberov SA. Molecular chemotherapy of pancreatic cancer using novel mutant bacterial cytosine deaminase gene. *Mol Cancer Ther* 2008;7:2845–2854.

Kam BL, Teunissen JJ, Krenning EP, de Herder WW, Khan S, van Vliet EI, *et al*. Lutetium-labelled peptides for therapy of neuroendocrine tumours. *Eur J Nucl Med Mol Imaging* 2012;39 Suppl 1:S103–112.

Kim HJ, Jeon YH, Kang JH, Lee YJ, Kim KI, Chung HK, *et al*. In vivo long-term imaging and radioiodine therapy by sodium-iodide symporter gene expression using a lentiviral system containing ubiquitin C promoter. *Cancer Biol Ther* 2007;6:1130–1135.

Kim KY, Kim SU, Leung PC, Jeung EB, Choi KC. Influence of the prodrugs 5-fluorocytosine and

CPT-11 on ovarian cancer cells using genetically engineered stem cells: tumor-tropic potential and inhibition of ovarian cancer cell growth. *Cancer Sci* 2010;101:955–962.

Kogai T, Kanamoto Y, Che LH, Taki K, Moatamed F, Schultz JJ, *et al*. Systemic retinoic acid treatment induces sodium/iodide symporter expression and radioiodide uptake in mouse breast cancer models. *Cancer Res* 2004;64: 415–422.

Kogai T, Kanamoto Y, Li AI, Che LH, Ohashi E, Taki K, *et al*. Differential regulation of sodium/iodide symporter gene expression by nuclear receptor ligands in MCF-7 breast cancer cells. *Endocrinology* 2005;146: 3059–3069.

Kogai T, Brent GA, The sodium iodide symporter (NIS): regulation and approaches to targeting for cancer therapeutics. *Pharmacol Ther* 2012; 135:355–370.

Kumar M, Liu ZR, Thapa L, Chang Q, Wang DY, Qin RY. Antiangiogenic effect of somatostatin receptor subtype 2 on pancreatic cancer cell line: Inhibition of vascular endothelial growth factor and matrix metalloproteinase-2 expression in vitro. *World J Gastroenterol* 2004;10:393–399.

LaDuca H, Stuenkel AJ, Dolinsky JS, Keiles S, Tandy S, Pesaran T, *et al*. Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients. *Genet Med* 2014;16:830–837.

Lee YL, Lee YJ, Ahn SJ, Choi TH, Moon BS, Cheon GJ, *et al*. Combined radionuclide-chemotherapy and in vivo imaging of hepatocellular carcinoma cells after transfection of a triple-gene construct, NIS, HSV1-sr39tk, and EGFP. *Cancer Lett* 2010; 290:129–138.

Li ZS, Pan X, Xu GM, Cui L, Dai GR, Gong YF, *et al*. Killing effects of cytosine deaminase gene mediated by adenovirus vector on human pancreatic cancer cell lines in vitro. *Hepatobiliary Pancreat Dis Int* 2003;2: 147–151.

Lim SJ, Paeng JC, Kim SJ, Kim SY, Lee H, Moon DH. Enhanced expression of adenovirus-mediated sodium iodide symporter gene in MCF-7 breast cancer cells with retinoic acid treatment. *J Nucl Med* 2007;48:398–404.

Lv SQ, Zhang KB, Zhang EE, Gao FY, Yin CL, Huang CJ, *et al*. Antitumor efficiency of the cytosine deaminase/5-fluorocytosine suicide gene therapy system on malignant gliomas: an *in vivo* study. *Med Sci Monit* 2009;15: BR13–20.

Maggisano V, Puppin C, Celano M, D'Agostino M, Sponziello M, Micali S, *et al*. Cooperation of histone deacetylase inhibitors SAHA and valproic acid in promoting sodium/iodide symporter expression and function in rat Leydig testicular carcinoma cells. *Endocrine* 2014;45:148–152.

McNeish IA, Green NK, Gilligan MG, Ford MJ, Mautner V, Young LS, *et al*. Virus directed enzyme prodrug therapy for ovarian and pancreatic cancer using retrovirally delivered E. coli nitroreductase and CB1954. *Gene Ther* 1998;5:1061–1069.

Mitrofanova E, Unfer R, Vahanian N, Daniels W, Roberson E, Seregina T, *et al*. Rat sodium iodide symporter for radioiodide therapy of cancer. *Clin Cancer Res* 2004;10:6969–6976.

Moon DH, Lee SJ, Park KY, Park KK, Ahn SH, Pai

MS, *et al*. Correlation between 99mTc-pertechnetate uptakes and expressions of human sodium iodide symporter gene in breast tumor tissues. *Nucl Med Biol* 2001;28: 829–834.

Myers CE. The pharmacology of the fluoropyrimidines. *Pharmacol Rev* 1981;33: 1–15.

Nimesh S. Potential implications of nanoparticle characterization on in vitro and in vivo gene delivery. *Ther Deliv* 2012;3:1347–1356.

Nishiyama T, Kawamura Y, Kawamoto K, Matsumura H, Yamamoto N, Ito T, *et al*., Antineoplastic effects in rats of 5-fluorocytosine in combination with cytosine deaminase capsules. *Cancer Res* 1985;45: 1753–1761.

Ogris M, Wagner E. Targeting tumors with non-viral gene delivery systems. Drug Discov Today 2002;7:479–485.

Pan D. *In situ* (*in vivo*) gene transfer into murine bone marrow stem cells. *Methods Mol Biol* 2009;506:159–169.

Ponomarev V, Doubrovin M, Serganova I, Beresten T, Vider J, Shavrin A, *et al*. Cytoplasmically retargeted HSV1-tk/GFP reporter gene mutants for optimization of noninvasive molecular-genetic imaging. *Neoplasia* 2003;5:245–254.

Ramnaraine M, Pan W, Goblirsch M, Lynch C, Lewis V, Orchard P, *et al*. Direct and bystander killing of sarcomas by novel cytosine deaminase fusion gene. *Cancer Res* 2003;63: 6847–6854.

Ray P, Wu AM, Gambhir SS. Optical bioluminescence and positron emission tomography imaging of a novel fusion reporter gene in tumor xenografts of living mice. *Cancer Res* 2003;63:1160–1165.

Ray P, De A, Min JJ, Tsien RY, Gambhir SS. Imaging tri-fusion multimodality reporter gene expression in living subjects. *Cancer Res* 2004; 64:1323–1330.

Renier C, Yao C, Goris M, Ghosh M, Katznelson L, Nowles K, *et al*. Endogenous NIS expression in triple-negative breast cancers. *Ann Surg Oncol* 2009;16:962–968.

Riesco-Eizaguirre G, De la Vieja A, Rodriguez I, Miranda S, Martin-Duque P, Vassaux G, *et al*. Telomerase-driven expression of the sodium iodide symporter (NIS) for in vivo radioiodide treatment of cancer: a new broad-spectrum NIS-mediated antitumor approach. *J Clin Endocrinol Metab* 2011;96:E1435–1443.

Ruggiero A, Brader P, Serganova I, Zanzonico P, Cai S, Lipman NS, *et al*. Different strategies for reducing intestinal background radioactivity associated with imaging HSV1-tk expression using established radionucleoside probes. *Mol Imaging* 2010;9:47–58.

Salomon B, Maury S, Loubiere L, Caruso M, Onclercq R, Klatzmann D. A truncated herpes simplex virus thymidine kinase phosphorylates thymidine and nucleoside analogs and does not cause sterility in transgenic mice. *Mol Cell Biol* 1995;15:5322–5328.

Schaer JC, Waser B, Mengod G, Reubi JC. Somatostatin receptor subtypes sst1, sst2, sst3 and sst5 expression in human pituitary, gastroentero-pancreatic and mammary tumors: comparison of mRNA analysis with receptor autoradiography. *Int J Cancer* 1997;70: 530–537.

Searle PF, Chen MJ, Hu L, Race PR, Lovering AL, Grove JI, *et al*. Nitroreductase: a prodrug-

activating enzyme for cancer gene therapy. *Clin Exp Pharmacol Physiol* 2004;31:811–816.

Serganova I, Mayer-Kukuck P, Huang R, Blasberg R. Molecular imaging: reporter gene imaging. *Handb Exp Pharmacol* 2008;167–223.

Shimura H, Haraguchi K, Miyazaki A, Endo T, Onaya T. Iodide uptake and experimental 131I therapy in transplanted undifferentiated thyroid cancer cells expressing the Na+/I- symporter gene. *Endocrinology* 1997;138:4493–4496.

Sollini M, Erba PA, Fraternali A, Casali M, Di Paolo ML, Froio A, *et al*. PET and PET/CT with 68gallium-labeled somatostatin analogues in Non GEP-NETs Tumors. Scientific World Journal 2014;2014:194123.

Sowa-Staszczak A, Pach D, Kunikowska J, Krolicki L, Stefanska A, Tomaszuk M, *et al*. Efficacy and safety of 90Y-DOTATATE therapy in neuroendocrine tumours. *Endokrynol Pol* 2011;62:392–400.

Spitzweg C, Dietz AB, O'Connor MK, Bergert ER, Tindall DJ, Young CY, *et al*. *In vivo* sodium iodide symporter gene therapy of prostate cancer. *Gene Ther* 2001;8:1524–1531.

Strum JM, Phelps PC, McAtee MM. Resting human female breast tissue produces iodinated proteins. *J Ultrastruct Res* 1983;84:130–139.

Tanosaki S, Ikezoe T, Heaney A, Said JW, Dan K, Akashi M, *et al*. Effect of ligands of nuclear hormone receptors on sodium/iodide symporter expression and activity in breast cancer cells. *Breast Cancer Res Treat* 2003;79: 335–345.

Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, *et al*. The mammary gland iodide transporter is expressed during lactation and in breast cancer. *Nat Med* 2000;6: 871–878.

Thorek D, Robertson R, Bacchus WA, Hahn J, Rothberg J, Beattie BJ, *et al*. Cerenkov imaging - a new modality for molecular imaging. *Am J Nucl Med Mol Imaging* 2012;2:163–173.

Trujillo MA, Oneal MJ, Davydova J, Bergert E, Yamamoto M, Morris JC, 3rd. Construction of an MUC-1 promoter driven, conditionally replicating adenovirus that expresses the sodium iodide symporter for gene therapy of breast cancer. *Breast Cancer Res* 2009;11:R53.

Unterholzner S, Willhauck MJ, Cengic N, Schutz M, Goke B, Morris JC, *et al*. Dexamethasone stimulation of retinoic Acid-induced sodium iodide symporter expression and cytotoxicity of 131-I in breast cancer cells. *J Clin Endocrinol Metab* 2006;91:69–78.

Wapnir IL, van de Rijn M, Nowels K, Amenta PS, Walton K, Montgomery K, *et al*. Immuno-histochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. *J Clin Endocrinol Metab* 2003;88:1880–1888.

Wapnir IL, Goris M, Yudd A, Dohan O, Adelman D, Nowels K, *et al*. The Na+/I- symporter mediates iodide uptake in breast cancer metastases and can be selectively down-regulated in the thyroid. *Clin Cancer Res* 2004; 10:4294–4302.

Warrell RP, Jr. Retinoid resistance in acute promyelocytic leukemia: new mechanisms, strategies, and implications. *Blood* 1993;82: 1949–1953.

Weedon SJ, Green NK, McNeish IA, Gilligan MG, Mautner V, Wrighton CJ, *et al*. Sensitisation of human carcinoma cells to the prodrug CB1954 by adenovirus vector-mediated expression of *E.*

coli nitroreductase. *Int J Cancer* 2000;86: 848–854.

Wei S, Yang J, Lee SL, Kulp SK, Chen CS. PPARgamma-independent antitumor effects of thiazolidinediones. *Cancer Lett* 2009;276: 119–124.

Willhauck MJ, DJ OK, Wunderlich N, Goke B, Spitzweg C. Stimulation of retinoic acid-induced functional sodium iodide symporter (NIS) expression and cytotoxicity of (1)(3)(1)I by carbamazepine in breast cancer cells. *Breast Cancer Res Treat* 2011;125:377–386.

Witlox MA, Lamfers ML, Wuisman PI, Curiel DT, Siegal GP. Evolving gene therapy approaches for osteosarcoma using viral vectors: review. *Bone* 2007;40:797–812.

Xu Y, Liu H, Chang E, Jiang H, Cheng Z. Cerenkov Luminescence Imaging (CLI) for cancer therapy monitoring. *J Vis Exp* 2012;e4341.

Yang XP, Liu L, Wang P, Ma SL. Human Sulfatase-1 Improves the Effectiveness of Cytosine Deaminase Suicide Gene Therapy with 5-Fluorocytosine Treatment on Hepatocellular Carcinoma Cell Line HepG2 and. *Chin Med J (Engl)* 2015;128:1384–1390.

Yin HY, Zhou X, Wu HF, Li B, Zhang YF. Baculovirus vector-mediated transfer of NIS gene into colon tumor cells for radionuclide therapy. *World J Gastroenterol* 2010;16: 5367–5374.

Zarogoulidis P, Darwiche K, Sakkas A, Yarmus L, Huang H, Li Q, *et al*. Suicide Gene Therapy for Cancer - Current Strategies. *J Genet Syndr Gene Ther* 2013;4.