Human sodium iodide transporter gene-mediated imaging and therapy of mouse glioma, comparison between ¹⁸⁸Re and ¹³¹I

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Abstract. Novel treatment options are urgently required for patients with glioma who are not effectively treated through standard therapy. Human sodium iodide symporter (hNIS) is a molecular target of certain tumors types. Compared with ¹³¹I, ¹⁸⁸Re possesses a higher energy and shorter half-life; therefore, the effects of ¹⁸⁸Re and ¹³¹I were compared in hNIS-mediated gene imaging and therapy in the present study. Recombinant human brain glioma cell line U87 was transfected with a recombinant lentiviral vector containing hNIS (U87-hNIS). U87-0 cell line transfected with blank lentivirus was prepared as a control. In vitro, the ¹⁸⁸Re and ¹³¹I uptake of U87-hNIS cells were 21.3-times and 25.9-times that of the control groups, however the excretion rate of the two nuclides was very rapid, and the half-life was only ~4 min. Sodium perchlorate inhibited hNIS-mediated ¹⁸⁸Re and ¹³¹I uptake to levels observed in the control groups. ¹⁸⁸Re and ¹³¹I were able to kill U87-hNIS cells selectively, with a survival of only 21.6 and 36.2%, respectively. U87-hNIS nude mice appeared to accumulate ¹⁸⁸Re, with a ratio of radioactivity counts between tumor and non-tumor sites of ~13.5 compared with 10.3 of ¹³¹I 1 h after radionuclide injection. In contrast with in vitro studies, U87-hNIS cells demonstrated a notable increase in ¹⁸⁸Re retention in vivo, even 24 h after ¹⁸⁸Re injection. U87-hNIS cells also exhibited increased ¹³¹I retention in vivo; however, as the time increased, ¹³¹I was rapidly released with the tumor no longer able to be imaged 24 h after ¹³¹I injection. Following treatment, U87-hNIS tumors experienced a volume reduction of 24.1%, whereas U87-0 cells demonstrated an increase of 28.8%. ¹⁸⁸Re and ¹³¹I were revealed to be effective at decreasing tumor volume compared with the control. However, ¹⁸⁸Re was significantly more potent compared with ¹³¹I (P<0.01). The present study indicated that the U87-hNIS cell line is sufficient to induce specific ¹⁸⁸Re and ¹³¹I uptake, which may kill cells

Key words: sodium iodide symporter, U87, ¹⁸⁸Re, ¹³¹I, therapy

in vitro and *in vivo*. ¹⁸⁸Re exhibited an increased retention time *in vivo* compared with ¹³¹I, which facilitates the imaging and therapy of U87-hNIS tumors.

Introduction

Glioma is one of the most common malignant tumor types globally with high morbidity and mortality. To date, surgical resection and radiotherapy remain the primary methods used for the treatment of glioma (1,2). Radiotherapy requires at least 55 Gy of external radiation to control the tumor, however, a radiation dose of >60 Gy often results in normal brain tissue necrosis (3-5). Therefore, novel therapeutic strategies are required.

Sodium iodide symporter (NIS) is a type of cell membrane glycoprotein that mediates the active uptake of iodine in the thyroid and other types of tissues (6). Gene therapy is an important alternative for the treatment of tumors; for example, radioiodine treatment of extra-thyroidal cancer via the ectopic transfer of the NIS gene into otherwise non-NIS-expressing cancer. A number of studies have successfully obtained ectopic expression of NIS in different tumors through gene transfer (7-10). The dual function of NIS as a diagnostic and therapeutic gene thereby allows easy monitoring of functional NIS expression using y camera imaging techniques prior to the application of a therapeutic radionuclide dose (11). Our previous studies (12-15) demonstrated that baculovirus-mediated human NIS (hNIS) expression may mediate multiple tumor uptake of ¹³¹I, providing a promising target for gene therapy. In previous studies, extra-thyroidal tissues have generally not been able to mediate iodide organification following NIS gene transfer, therefore rapid outflow of ¹³¹I resulted in a decreased radiation dose able to affect the activity of tumor cells, thus decreasing the curative effect (16). The use of alternative NIS-transported radioisotopes with a higher energy than ¹³¹I may improve the efficacy of NIS-mediated radionuclide targeted therapy (17,18). ¹⁸⁸Re is a type of short physical half-life radionuclide (0.71 days), which has been applied to treat various diseases, including prostate carcinoma, hepatoma carcinoma, breast carcinoma, bladder carcinoma, refractory arthritis and coronary beta-brachytherapy (19-22). Compared with ¹³¹I (0.192 MeV), due to its high energy, ¹⁸⁸Re (23) is an ideal therapeutic alternative (0.778 MeV). Our previous study (24) suggested that lentiviruses mediate hNIS expression

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that results in ¹⁸⁸Re uptake in glioma and provides a promising gene therapy strategy. However, the physical half-life of ¹⁸⁸Re is shorter (0.71 days) compared with that of ¹³¹I (8.02 days). Therefore, the purpose of the present study was to compare the therapeutic effects of ¹⁸⁸Re- and ¹³¹I-mediated gene therapy *in vitro* and *in vivo*.

Materials and methods

Plasmid construction, lentivirus preparation and U87 cell transfection with lentivirus. Human NIS gene was obtained in the form of a pcDNA3.1-hNIS plasmid (provided by Dr Sissy Jhiang from Ohio University, OH, USA). U87 glioma cell line [American Type Culture Collection (ATCC), Manassas, VA, USA] was maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.; 5% CO₂, 37°C). The plasmid containing hNIS was packaged into lentivirus vector (Thermo Fisher Scientific, Inc.) and named as Lenti-CMV-hNIS. To generate cell line expressing hNIS controlled by cytomegalovirus-enhancer/promoter, Lenti-CMV-hNIS was added to U87 cells at a multiplicity of infection of 2.0, then were selected for further study after 3 weeks. The transfected cell line stably expressing hNIS was named U87-hNIS. U87-hNIS was validated by radionuclide uptake experiments. Cells were inoculated with DMEM containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μ g/ml streptomycin in 24-well plates 24 h prior to the experiment to achieve a density of 10⁵ cells per well at the day of experiment.

In vitro radioisotope uptake experiments. ¹⁸⁸Re was eluted from a ¹⁸⁸W/¹⁸⁸Re generator (Jiangsu ReTai Pharmaceutical Biotechnology Co., Ltd., Changzhou, China) using 0.9% saline.¹³¹I was supplied by Shanghai Kexin Biotech Co., Ltd. (Shanghai, China). In vitro 188 Re/131 uptake experiments of U87-hNIS cells expressing hNIS were performed as Weiss and Grollman (25) described previously, with minor modifications. All data were corrected for attenuation. U87-hNIS cells were washed with 0.5 ml buffered Hanks' balanced salt solution (bHBSS; containing 10 µM HBSS and buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.3), incubated in 0.5 ml bHBSS (containing 3.7 kBq ¹⁸⁸Re/¹³¹I) liquid for 1, 2, 5, 10, 20, 30, 60 and 120 min respectively, then washed two times with ice-cold bHBSS, incubated with 1 ml of 100% dehydrated ethanol for 20 min, followed by cell lysis (using trypsin and 0.25% EDTA; Gibco; Thermo Fisher Scientific, Inc.). Finally, the radioactivity (count per min) was detected using a well gamma-counter (Shanghai Institute of Nuclear Research Rihuan Instrument Co., Shanghai, China) and compared with background, in order to achieve a ratio. All the following experiments were conducted in triplicate.

Sodium perchlorate inhibition study. The specificity of ¹⁸⁸Re/¹³¹I uptake was investigated in the present study. U87-hNIS cells (at a density of 10⁵) were incubated with sodium perchlorate (Sigma-Aldrich; Merck KGaA, Darmstadt,

Germany) and 3.7 kBq ¹⁸⁸Re/¹³¹I in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μ g/ml streptomycin (5% CO₂, 37°C) for 30 min, in which the concentration of sodium perchlorate was 0, 1, 2, 5, 10, 20, 50 and 100 μ M, respectively. Thereafter, the cells were washed with bHBSS twice, lysed (using trypsin and 0.25% EDTA; Gibco; Thermo Fisher Scientific, Inc.) at room temperature for three min, and their radioactivity measured, according to the methods aforementioned.

Radioisotope efflux study in vitro. The ¹⁸⁸Re/¹³¹I efflux kinetics of U87-hNIS cells were investigated as previously described (25). U87-hNIS cells (at a density of 10^5) were incubated with ¹⁸⁸Re/¹³¹I (3.7 kBq/well) at 37°C for ~30 min. The culture medium (DMEM containing 10% fetal bovine serum) was then replaced with nonradioactive bHBSS. Cells were incubated for 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 min and immediately lysed using trypsin and 0.25% EDTA (Gibco; Thermo Fisher Scientific, Inc.). Subsequently, cells were extracted with 1 ml dehydrated alcohol and the radioactivity in the cells was measured as aforementioned.

In vitro assessment of radioisotope toxicity by clonogenic assay. Prior to the experiment, U87-hNIS cells were cultured in 24-well plates, in order to achieve a density of 10⁵ cells/well at the day of study. As presented in Table I, cells were divided into five groups. Group 1 comprised U87-hNIS cells washed with bHBSS with 740 kBq¹⁸⁸Re, then cultured (5% CO₂, 37°C) for 7 h. Group 2 was processed much the same as group 1, with the exception that ¹⁸⁸Re was replaced with ¹³¹I. Groups 3 and 4 were non-transfected U87 cells with ¹⁸⁸Re and ¹³¹I, respectively. Group 5 comprised non-transfected U87 cells without the addition of any radionuclide. Groups 3, 4 and 5 all served as controls. The 5 groups of cells were then washed and lysed as described above, and seeded in 6-well plates at a density of 200 cells/well. The cells were cultured for 7 days (5% CO₂ at 37°C). Following removal of the culture medium as aforementioned, cells were stained with crystal violet solution (0.1%) at room temperature for 10 min and the colonies >30 cells were counted using an optical microscope (x100 magnification; CKX41; Olympus Corporation, Tokyo, Japan). Results were expressed as the percentage of surviving cells compared with untreated U87 cells (Group 5).

Animal models. A total of 18 female 5-week-old athymic Balb/c nude mice (18-20 g in weight) were used (Shanghai Laboratory Animal Centre Chinese Academy of Sciences, Shanghai, China). A full light-dark cycle with 10 h of light and 14 h of darkness each day was applied, with conditions including a temperature of 26-28°C, a humidity of 40-60%, and general feed and drinking water provided. Mice were sacrificed 4 weeks after the first intravenous injections of ¹⁸⁸Re or ¹³¹I. Animal experiments were approved by the Ethics Committee and Animal Care Committee of Shanghai JiaoTong University, School of Medicine (Shanghai, China). A xenograft model was generated by subcutaneous injection of U87-hNIS cells (5x10⁶ cells, suspended in 150 µl PBS) into the right armpit of the mice. Subsequently, ~6 weeks following inoculation, the tumor diameters reached 0.8-1.0 cm, which could be used for imaging and therapies.

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Characteristic	Group 1	Group 2	Group 3	Group 4	Group 5
U87-human sodium iodide					
symporter U87	+	+	+	+	+
¹⁸⁸ Re	+		+	I	
¹³¹ I		+		+	



Tumor imaging and therapy study in a xenograft model. Tumor imaging studies were performed in six U87-hNIS nude mice. ¹⁸⁸Re (55.5 MBq) or ¹³¹I (55.5 MBq) was injected through the caudal vein. Tumor images were performed 15 min, 1, 2, 4 and 24 h after injection using a high-resolution gamma camera equipped with a pinhole collimator (GE Healthcare Life Sciences, Little Chalfont, UK) immediately following ¹⁸⁸Re or ¹³¹I injection. Each image lasted for 3 min. The matrix size was 256x256. Region of interest was placed on the area of tumor and the contralateral axillary, and the ratio of radioactivity counts between tumor and non-tumor sites (T/NT) at each time point was measured.

Tumor therapy studies were performed in U87-hNIS nude mice at the same time. In the present study, no animal presented with multiple tumors, and when the tumor diameter of nude mice grew to 0.8-1.0 cm in diameter (the minimum and maximum diameter exhibited by a single tumor in the present study were 0.82 and 0.96 cm, respectively), they were randomly divided into three groups (n=6). Group 1 was given two intravenous injections of 55.5 MBq ¹⁸⁸Re with an interval of 1 week. Group 2 was treated intravenously with 2 injections of 55.5 MBq ¹³¹I with a 1 week interval. Group 3 was left untreated. In order to evaluate the therapeutic effect of hNIS mediated radionuclides, tumor size was measured with calipers prior to and following the administration of ¹⁸⁸Re or ¹³¹I every week, in three dimensions. Tumor volume was estimated by the following formula: Length x width²x0.52.

Statistical methods. Origin 7.5 (OriginLab, Northampton, UK) and SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) software were used in the present study. All experiments were performed in triplicates unless otherwise indicated. Numeric data are expressed as mean \pm standard deviation. One-way analysis of variance was performed to evaluate the difference amongst groups followed by Tukeys post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Preparation of the lentiviruses. The lentiviral vector Lenti-cytomegalovirus (CMV)-hNIS containing CMV promoter and hNIS gene was packed successfully, and the Lenti-CMV-0 vector was packed as control. Stable cell lines U87-hNIS and U87-0 were established 3 weeks after infection with recombinant lentivirus. For the purpose of comparing hNIS mediated uptake of radioisotope in the cell

Figure 1. Effect of time on the ¹⁸⁸Re and ¹³¹I uptake ratio (compared with background) in U87-hNIS cells. The ratio was measured following incubation for various periods of time with 3.7 kBq of ¹⁸⁸Re and ¹³¹I in buffered Hanks' balanced salt solution. Data are presented as the mean \pm standard deviation (n=3). hNIS, human sodium iodide symporter.

line, ¹⁸⁸Re and ¹³¹I uptake experiments were performed and compared in the following studies.

hNIS mediated in vitro radioisotope uptake. In U87-hNIS cells, uptake of ¹⁸⁸Re/¹³¹I in relation to incubation time is presented in Fig. 1. The results reveal the initial ¹⁸⁸Re/¹³¹I uptake dependent on incubation time. The results demonstrated that ¹⁸⁸Re/¹³¹I influx rapidly into U87-hNIS cells, reaching a peak at 30 min. The ¹⁸⁸Re/¹³¹I uptake of U87-hNIS cells was measured to be 21.3 and 25.9-fold higher compared with that of U87-0 cells. The radioactivity measured 2 h after ¹⁸⁸Re/¹³¹I incubation was 84.7 and 88.5% of the maximal uptake. ¹³¹I experienced an increased uptake compared with ¹⁸⁸Re in U87-hNIS cells. In a similar study performed by Zuckier *et al* (26), ¹⁸⁸Re and ¹³¹I were reported to demonstrate a high uptake.

Sodium perchlorate inhibition study. In order to verify the specificity of hNIS mediated ¹⁸⁸Re/¹³¹I uptake, ¹⁸⁸Re/¹³¹I inhibition test was performed in U87-hNIS cells in the presence of varying concentrations of sodium perchlorate, an already confirmed competitive inhibitor. Fig. 2 presents the effect of distinct concentrations of sodium perchlorate on ¹⁸⁸Re/¹³¹I uptake in U87-hNIS cells. When cells were treated with sodium perchlorate (1-100 μ M), ¹⁸⁸Re/¹³¹I uptake in U87-hNIS cells was inhibited in a dose-dependent manner. At 5 μ M, sodium perchlorate decreased ¹⁸⁸Re/¹³¹I uptake to 15.3 and 18.1% of original levels, respectively, compared with and an inhibition rate of 96 and 95.3% at 50 μ M. These results demonstrate that sodium perchlorate may specifically block ¹⁸⁸Re/¹³¹I uptake in U87-hNIS cells, suggesting that ¹⁸⁸Re/¹³¹I uptake in U87-hNIS cells is mediated by functional hNIS expression.

Radionuclide efflux assay. ¹⁸⁸Re/¹³¹I efflux assays were performed 30 min after the addition of ¹⁸⁸Re/¹³¹I into the cultured cells (the aforementioned cell uptake experiment identified that the highest intracellular uptake was obtained at 30 min). The culture medium containing ¹⁸⁸Re/¹³¹I was replaced by nonradioactive DMEM, the remaining ¹⁸⁸Re/¹³¹I activity in U87-hNIS cell lysate was determined. As presented in Fig. 3, the intracellular radionuclides were released rapidly



Figure 2. Sodium perchlorate inhibition study. Sodium perchlorate was added at the indicated concentrations (0, 1, 2, 5, 10, 20, 50 and 100 μ M), ¹⁸⁸Re and ¹³¹I uptake was measured as a ratio compared with background. Data are presented as the mean ± standard deviation (n=3).



Figure 3. Time dependent ¹⁸⁸Re and ¹³¹I efflux assay in U87-human sodium iodide symporter cells after 20 min incubation with 3.7 kBq ¹⁸⁸Re and ¹³¹I in 0.5 ml buffered Hanks' balanced salt solution.¹⁸⁸Re and ¹³¹I uptake was measured as a ratio compared with background. Data are presented as the mean \pm standard deviation (n=3).

into the medium, with >50% of radionuclides excreted out of the cells within the first 4 min, whereas a limited amount of residual radionuclide was observed 20 min after ¹⁸⁸Re/¹³¹I was replaced by nonradioactive DMEM. These results suggested that radioactivity is rarely retained in U87-hNIS cells without reuptaking.

Radio toxicity assessed by colony-forming assay. Following treatment with ¹⁸⁸Re/¹³¹I, a colony-forming experiment was performed to calculate the survival rate of the cells. The results are presented in Fig. 4. The data are expressed as the percentage of surviving cells. In Groups 3 and 4, U87-0 cells were treated with ¹⁸⁸Re/¹³¹I, with the number of colonies representing 76.3 and 85.7% of the blank control group (Group 5), indicating that the two radionuclides exhibit nonspecific killing effects on U87-0 cells. Following treatment with ¹⁸⁸Re/¹³¹I, survival of Groups 1 and 2 were markedly decreased



Figure 4. Survival rates of U87-hNIS cells treated with ¹⁸⁸Re/¹³¹I. ¹⁸⁸Re/¹³¹I toxicity was assessed using a colony-forming assay. Cells were divided into 5 groups and treated as described on the x-axis. Clones of >30 cells were counted. Results were expressed as the percentage of surviving clones obtained following treatment with ¹⁸⁸Re/¹³¹I. U87, U87+¹³¹I and U87+¹⁸⁸RE served as controls. Data are presented as the mean ± standard deviation (n=3). hNIS, human sodium iodide symporter.

compared with that of the other 3 groups, with survival rates of only 21.6 and 36.2%, respectively. These results suggest that ¹⁸⁸Re/¹³¹I exhibits a selective killing effect on U87-hNIS cells, with an increased killing capacity of ¹⁸⁸Re compared with that of ¹³¹I.

Tumor imaging and therapy study. The results of in vivo uptake and efflux of ¹⁸⁸Re/¹³¹I were different from those observed in vitro. U87-hNIS tumor demonstrated an efficient ¹⁸⁸Re/¹³¹I uptake *in vivo*, which accumulated ¹⁸⁸Re/¹³¹I rapidly and significantly as indicated by the arrows in imaging results of the right armpit in Fig. 5. Organs naturally expressing hNIS (such as the thyroid and stomach) and organs participating in ¹⁸⁸Re/¹³¹I excretion (such as the kidneys and bladder) were able to be visualized. Quantified results are presented in Fig. 6. The accumulation of ¹⁸⁸Re/¹³¹I reached a peak 1 h after administration (T/NT, 13.5±3.2 and 10.3±2.1, respectively). ¹⁸⁸Re remained at a steady level until 24 h (T/NT, 11.6±2.1), whereas ¹³¹I experienced limited retention at 24 h (T/NT, 3.9 ± 0.7). Thyroid naturally expressing NIS markedly accumulated ¹³¹I 24 h after injection, whereas ¹⁸⁸Re demonstrated limited accumulation (Fig. 5). As a result of lacking organification, ¹⁸⁸Re cannot be retained in the thyroid for a prolonged period of time, therefore its half-life is relatively short in the thyroid and may protect the thyroid from hypothyroidism. In contrast, ¹³¹I can be organified by the thyroid, which alongside a long half-life may increase the risk of hypothyroidism.

A total of 3 weeks following treatment, a difference in tumor volume was demonstrated among mice treated with ¹⁸⁸Re (409.6±113.4 mm³), ¹³¹I (468.4±148.9 mm³) and non-treated animals (582.1±178.8 mm³) (P<0.05). No difference was observed between the ¹⁸⁸Re and ¹³¹I treatment group at 3 weeks. The association between treatment group and tumor volume was more significant at 4 weeks post treatment. The tumor volume was significantly different between mice treated with ¹⁸⁸Re (317.2±72.1 mm³), ¹³¹I (560.8±97.4 mm³) and non-treated (861.1±153.8 mm³) (P<0.01; Fig. 7). Following therapy, ¹⁸⁸Re treated U87-hNIS tumors experienced a volume



Figure 5. Scintigraphic images of nude mice treated with ¹⁸⁸Re (top row) and ¹³¹I (bottom row). Mice were imaged with 55.5 MBq ¹⁸⁸Re/¹³¹I.



Figure 6. Time dependent T/NT of ¹⁸⁸Re^{/131}I uptake by U87-human sodium iodide symporter tumor bearing nude mice. Data are presented as the mean \pm standard deviation (n=6). T/NT, ratio of radioactivity counts between tumor and non-tumor sites.



Figure 7. ¹⁸⁸Re/¹³¹I decreased tumor growth in U87-hNIS tumor bearing nude mice, compared with control. 55.5 MBq of ¹⁸⁸Re/¹³¹I was injected intravenously into each mouse. The tumor size was measured prior to and following the administration of ¹⁸⁸Re/¹³¹I. Data are presented as the mean ± standard deviation (n=6). hNIS, human sodium iodide symporter. *P<0.05 and **P<0.01 with comparisons indicated by lines.

reduction of 24.1%, whereas ¹³¹I treated U87-hNIS tumors demonstrated a volume increase of 28.8%. ¹⁸⁸Re and ¹³¹I were

demonstrated to be effective at decreasing the tumor volume compared with the untreated control, with ¹⁸⁸Re revealed to exhibit an increased effect compared with ¹³¹I (P<0.01).

Discussion

NIS-mediated radionuclide therapy possesses several advantages (27), making it an attractive approach for glioma imaging and therapies. It does not require complex radiolabeling procedures, it also is small-sized allowing it to penetrate the blood brain barrier and diffuse into the tumor (28). Radioisotopes exhibit the potential of a bystander effect, which may destroy tumor cells without hNIS expression by radionuclides emitted from surrounding hNIS expression tumor cells (29). The hNIS gene is not expressed in normal cells however is in glioma cells (30), this indicates that it may assist in improving the specificity of tumor therapy and decreasing the damage to normal brain tissue.

¹³¹I is an isotope commonly used in NIS-associated radionuclide therapy studies (31). However, as the radiation energy is not high enough, the rapeutic efficacy of 131 I is limited (3). The use of a high-energy radionuclide also transported by NIS including ¹⁸⁸Re, may be an effective strategy to improve therapeutic effect. A previous study demonstrated that ¹⁸⁸Re and ¹²⁵I share similar biodistribution patterns in mice, with the exception of the thyroid gland, as the thyroid gland has an organic function of iodine, which can retain ¹²⁵I (26). With lower γ -photons energy, ¹⁸⁸Re (155 keV) (32) is suitable for imaging, unlike ¹³¹I (364 keV). ¹⁸⁸Re can be easily generated using a ¹⁸⁸W/¹⁸⁸Re generator (33). These attractive physical properties make ¹⁸⁸Re a promising candidate for tumor imaging and therapy (19). ¹⁸⁸Re possesses a higher β energy, but relatively shorter physical half-life compared with ¹³¹I (34). Therefore, in the current study, the effects of ¹⁸⁸Re and ¹³¹I in the treatment of glioma were compared in vivo and in vitro.

In the present study, it was demonstrated that a lentivirus containing CMV-hNIS expression cassette is able to transfect U87 cells, as revealed by efficient ¹⁸⁸Re/¹³¹I uptake in U87-hNIS cells. ¹⁸⁸Re/¹³¹I exhibit rapid uptake in U87-hNIS cells, and the dynamic uptake pattern of ¹⁸⁸Re/¹³¹I was similar to that of previous studies (30,31). ¹⁸⁸Re uptake of U87-hNIS was up to 21.3-times that of the U87-0 control group, whereas ¹³¹I uptake was up to 25.9-fold of that of the control group. The two isotopes experienced a plateau phase akin to previous studies (35,36), when the uptake and efflux of the isotopes reached a state of equilibrium (25). As with previous NIS studies, radionuclide retention time was short, and rapid outflow remained a problem in the *in vitro* studies, in the present study. Neither ¹⁸⁸Re nor ¹³¹I experienced a long retention time *in vitro*.

In the *in vitro* experiments, isotope flow was rapid, with the outflow kinetics of the two radionuclides being similar. In addition, the colony formation assay demonstrated that U87-hNIS cells could be killed effectively by ¹⁸⁸Re and ¹³¹I. The absorbed dose of ¹⁸⁸Re was sufficiently high to selectively kill 78.4% of the cells, whereas ¹³¹I was able to kill up to 63.8% of the cells. These data are sufficient to suggest that even with a lower uptake than that of ¹³¹I, ¹⁸⁸Re may also be able to deliver a greater radiation dose to tumor cells, which is similar to the results reported by Kang et al (37) who identified that ¹⁸⁸Re uptake increased 87-times, whereas ¹²⁵I uptake increased 150-times in NIS transfected hepatocellular carcinoma cells, compared with untransfected cells. Following treatment with 188 Re or 131 I, the survival rate of cells was 28.9 and 46.3% respectively. Therefore, a superior therapeutic effect of ¹⁸⁸Re was demonstrated.

In the *in vitro* study, cells in the culture dishes were arranged in a single layer, which is not a three-dimensional structure. Conclusions from the cells cannot be applied to solid glioma with a three-dimensional structure, which requires further confirmation in xenografts. In vivo, U87-hNIS glioma cells are close to each other, which allows for rapid reuptake of radionuclides in the cells and serves as a mechanism for isotope trapping by glioma. The results of the in vivo imaging investigations in the present study suggested that ¹⁸⁸Re remained in U87-hNIS glioma cells even 24 h after administration, whereas a limited amount of ¹³¹I was retained in the cells 24 h after treatment. The results of the present study suggest that ¹⁸⁸Re cannot be retained in the thyroid gland, as it cannot be organified. A previous study has reported a similar biodistribution pattern for ¹³¹I and ¹⁸⁸Re (38). In fact, the thyroid will decrease the competitive uptake of ¹⁸⁸Re, thereby decreasing thyroid injury and increasing tumor reuptake of ¹⁸⁸Re, which is an advantage of hNIS-mediated radionuclide therapy in non-thyroid tumors. In addition, the results of the present study demonstrated that the degree of ¹⁸⁸Re/¹³¹I uptake and retention are sufficient to achieve tumor therapeutic effects. Following treatment for 4 weeks, there was a significant difference in tumor size between ¹⁸⁸Re treated U87-hNIS mice and ¹³¹I treated U87-hNIS mice.

The present study also possessed a number of limitations. A stable hNIS transfected cell line was used to express hNIS, which could not be used directly in clinical practice. As a result of the variation of hNIS transfection efficiency and the difference in specificity, actual clinical efficacy of ¹⁸⁸Re and ¹³¹I requires further evaluation. It is reported that U87 MG ATCC cell line has been contaminated/misidentified, however it is most probably also a glioblastoma cell line (39). hNIS mediates uptake of iodine in thyroid and numerous types of non-thyroid cells (6), as a result, a number of previous studies have also successfully obtained ectopic expression of hNIS in different tumors by gene transfer (7-10). The main purpose of

the present study was to compare the therapeutic effects of ¹⁸⁸Re and ¹³¹I mediated by hNIS, rather than a study into the underlying molecular mechanisms of their effects, therefore the U87 cell line could be replaced with a variety of cell lines that express hNIS, as such the aforementioned misidentification/contamination issue is unlikely to have affected the outcomes of the present study.

In conclusion, the results of the present study demonstrated that ¹⁸⁸Re appeared to be more efficient than ¹³¹I in the treatment of gliomas. The strategy appears to be a novel method for tumor imaging and therapy. However, improvements to decrease efflux and achieve increased radiation doses in the target tissue are still required.

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