Radioiodide uptake in melanoma cells after transfer of human NaI symporter gene

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Abstract To obtain human sodium/iodide symporter gene cDNA for studying its potential ability as a radioiodide treatment for melanoma, the hNIS gene cDNA was amplified with total RNA from human thyroid tissue by RT-PCR. The hNIS cDNA was inserted into cloning vector pUCm-T and subcloned into eukaryotic expression vector pc-DNA$_3$. The pc-DNA$_3$-hNIS and pc-DNA$_3$ were transduced into melanoma cells (B16) by electroporation, and two cell lines termed B16-A and B16-B respectively were established. The uptake and efflux of iodide was examined in vitro. The three cell lines (B16-A, B16-B, B16) were injected subcutaneously into the right flank of C57 mice. Biodistribution study and tumor imaging were performed when the tumor reached approximately 10mm in diameter. The cloned hNIS cDNA sequence was identical with the published sequence. Two novel cell lines named 16-A containing pc-DNA$_3$-hNIS and B16-B containing pc-DNA$_3$ only were established. The resultant cell line B16-A accumulated 17 and 19 times more radioiodide in vitro than B16 and B16-B respectively. The iodide uptake reached the half-maximal level within 10 min, and reached a plateau at 30 min. The efflux of iodide was also rapid ($T_{1/2eff}$=10min). The imaging shows in vivo uptake in expected sites including the salivary glands, thyroid, stomach, and hNIS-transduced tumor, whereas the nontransduced tumor was not visualized. The %ID/g of B16-A tumors at 1, 2, 4, 12, and 24h after injection of ^{125}I were 12.22±0.71, 10.91±0.72, 8.73±0.99, 1.24±0.29, and 0.19±0.03, respectively, which were significantly higher percentages than those for controlling tumors, p<0.01. However, biologic $T_{1/2}$ was about 6 h. Our preliminary data indicate that the transduction of the hNIS gene per se is sufficient to induce iodide transport in melanoma cells both in vitro and in vivo, but $T_{1/2eff}$ is short.

Keywords Radioiodide, Human NaI symporter, Gene therapy, Melanoma

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1 Introduction

Radioiodide therapy using ^{131}I is effective for patients who have metastasis from differentiated thyroid cancer. This therapy is based on the uptake of radioactive iodide into cancer cells, as is observed in thyroid cells, to produce the thyroid hormones thyroxine and triiodothyroxin, which are essential for the growth, development, and metabolism of most tissues.

Recently, the sodium/iodide symporter (NIS) gene was cloned by Smanik *et al*,[1] and some reports have described transfection of the NIS gene into several nonthyroidal cells as well as into thyroidal cells to express symporter protein.[2-10] If the transfected cells take up and concentrate rodioiodide, radiotherapy with ^{131}I may be applicable to NIS-transfected cancer in the manner in which metastatic thyroid cancer is treated clinically.

Melanoma is not too rare and malignant. As the first step of a novel strategy to use radioiodide concentrator gene therapy for malignant tumors, this preliminary study was designed to observe transfected melanoma cells both in vitro and in vivo.

2 Materials and methods

2.1 Cloning of human NIS gene

The human NIS gene was prepared as follows. In brief, total RNA was extracted from human thyroid

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tissue using a MicroFast Track kit (Invitrogene, Carlsbad, CA). Reverse transcription was performed using an oligo-(dT) primer and avian myeloblastosis virus reverse transcriptase, and polymerase chain reaction using Taq polymerase and the following primers according to the reported sequence:[1] forward primer:5'-CCCAAGCTTGCCCTCATGGAGGCCGTG GAG-3'; reverse primer: 5'-TGCTCTAGAGGCTG GCCCTGTCCTCAGAGG-3'. The polymerase chain reaction product was directly subjected to TA cloning using the pUCm-T vector (Invitrogen). The complementary DNA (cDNA) clone obtained was confirmed by digestion with restriction enzymes (*Hin*dⅢ/*Xba* Ⅰ/*Pst*Ⅰ) and sequencing.

2.2 Cell culture and establishment of melanoma cell lines expressing NIS

B16 cells were cultured in RPMI1640 medium (GIBCO BRL, Grand Island, NY) supplemented with 5% calf serum. Human NIS cDNA was subcloned into eukaryotic expression vector pc-DNA_3, pc-DNA_3-hNIS and pc-DNA_3 were transduced into melanoma cells (B16) by electroporation. Stable transduced cells were selected by G418, and two cell lines termed B16-A which exhibited the highest iodide uptake and B16-B respectively were established.

2.3 Iodide uptake and efflux assay in vitro

The iodide uptake and efflux were performed according to the method of Weiss *et al*.[11] Cells were plated in 24-well plates and cultured with RPMI1640 medium containing 5% calf serum. When the cells reached confluence (approximately1×10^6 cells), $^{125}I^-$ uptake was examined. Iodide uptake was determined by incubating cells with 500μL Hank's balanced salt solution (HBSS) with 3.7 kBq carrier-free $Na^{125}I$ and 10 μmol/L NaI, to yield a specific activity of 740 MBq/mmol at 37 ºC for 5~120 min. After incubation, the cells were washed twice on ice as quick as possible (<15 s) with 2 mL ice-cold HBSS incubation buffer, which was void of iodide. The cells were detached with 400 μL of 1%Triton X-100, and radioactivity was quantified with a well gamma-counter. With regard to iodide efflux, the washed cells were incubated for 20 min at 37 ºC with 500 μL of HBSS containing 3.7 kBq carrier-free $Na^{125}I$ and 10 μmol/L NaI. The medium was then removed and replaced with 2mL ice-cold HBSS incubation buffer. After the last medium removal, the cells were extracted with 400 μL of 1%Triton X-100 to count residual radioactivities.

2.4 Preparation of subcutaneous xenografts in C57 mice

The B16, B16-A, and B16-B cell lines were cultured at 37 ºC in a humidified 5% CO_2 atmosphere using RPMI1640 supplemented with 5% fetal calf serum. Cells to be injected into C57 mice (3 weeks of age) were taken from the culture during the log phase of growth. Tumors were induced by subcutaneous injection of 200 μL of sterile PBS containing 5×10^5 cells in the right flank. When tumors had reached 10mm in diameter (approximately 2 weeks after cell injection), radioiodide uptake studies and tumor imaging were performed.

2.5 Analysis of iodide uptake in vivo

At 1, 2, 4, 12, and 24 h after intraperitoneal injection of 14.8 kBq $Na^{125}I$, the tumor-bearing mice (10 mice/phase/group) were killed and tumors were removed, weighted, and counted for radioactivity. Data were expressed as percentage of injected dose per gram of tissue (%ID/g). From these, the half-life ($T_{1/2}$) of $^{125}I^-$ release was calculated.

2.6 Tumor imaging and semi-quantitation

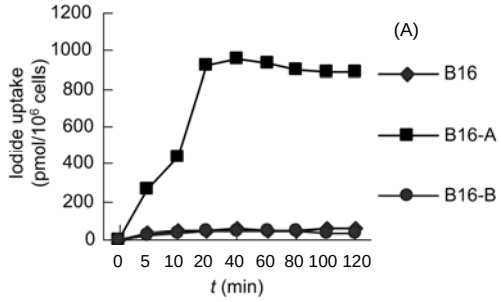
For the imaging of tumor-bearing mice, 7.4 MBq $Na^{131}I$ was administered intraperitoneally. Two hours after the injection, the mice were anesthetized by an intraperitoneal injection of sodium pentobarbital, and scintigrams were obtained using a gammar camera (TOSHIBA，GCA-901A/SA) equipped with a pinhole collimator. Semi-quantitation of uptaken radioiodide in tumors was performed using ROI technology.

The results were statistically analyzed using a *t* test for in vivo studies. Differences were considered significant when the probability value was less than 0.05.

3 Results

3.1 Time course of iodide uptake and iodide efflux

As shown in Fig.1A, the iodide uptake reached the half-maximal level within 10 min, and reached a plateau at 30 min. The plateaued, steady state seems to reflect a balance of influx and efflux of iodide. In addition, the iodide uptake in B16 and B16-B cells without transduction of human NIS gene were at the baseline level. Iodide efflux was studied for B16-A cells only (Fig.1B). The radioiodide that had accumulated in B16-A cells was rapidly excreted, with a $T_{1/2}$ of 10 min.



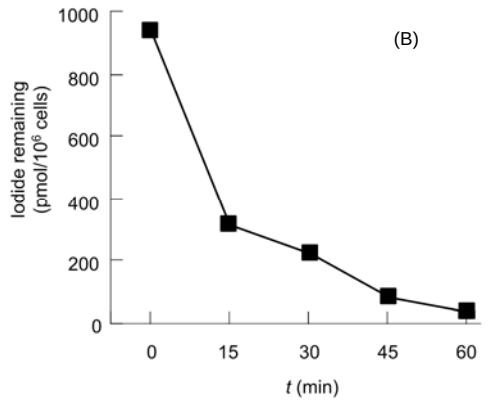


Fig.1 The course of iodide uptake by B16, B16-A, and B16-B cells (A) and iodide efflux from B16-A cells (B).

3.2 Accumulation of ^{125}I in tumors

The %ID/g of B16-A tumors at 1, 2, 4, 12, and 24 h after injection of ^{125}I were 12.22±0.71, 10.91±0.72, 8.73±0.99, 1.24±0.29 and 0.19±0.03, respectively, which were significantly higher percentages than those for controlling tumors, p<0.01 (Table 1). However, biologic $T_{1/2}$ was only about 6 h.

Table 1 Accumulation of ^{125}I in tumors

Phase (h)	ID%/g*		t (n=10)	p
	Experimental group (B16-A)	Control (B16-B+B16)		
1	12.22±0.71	2.34±0.26	40.14	<0.01
2	10.91±0.72	0.61±0.06	46.72	<0.01
4	8.73±0.99	0.33±0.03	27.90	<0.01
12	1.24±0.29	0.09±0.02	12.98	<0.01
24	0.19±0.03	0.02±0.01	16.68	<0.01

*All data are mean values ±SD

3.3 Tumor imaging and semi-quantitation

The images were consistent with the results of the ^{125}I accumulation data. B16-A tumors were visualized clearly (Fig.2A), but the B16 (Fig.2B) and B16-B (Fig.2C) tumors were not seen. Clear images were obtained as early as 2 h after injection of ^{131}I, but physiological uptake in the salivary gland, thyroid, and stomach was seen. The ratios of ^{131}I accumulated in tumors were 18.18 (B16-A group/B16-B group), 17.60 (B16-A group/B16 group), and 0.97 (B16-B group/B16 group), respectively.

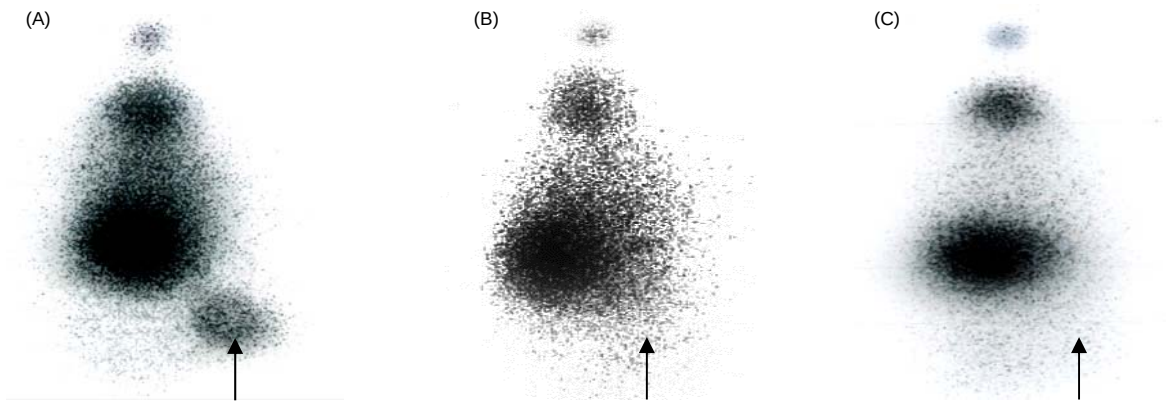


Fig.2 Typical images of tumor bearing C57 mice subcutaneously transplanted B16-A cells (A), B16-B cells (B), and B16 cells (C) in the right flank. ^{131}I imaging was completed after intraperitoneal injection of 7.4 MBq Na^{131}I. Scintigraphies were obtained 2 h after injection of ^{131}I using a gammar camera (TOSHIBA，GCA-901A/SA) equipped with a pinhole collimator.

4 Discussion

The recent cloning of the NIS gene is not only of major importance for the understanding of the mechanisms underlying iodide transport in the thyroid but also could open new therapeutic perspectives. Indeed, achievement of efficient transfer of NIS gene, coupled to radioactive iodide administration, may allow radioiodine treatment of nonthyroid tumors as well as thyroid tumors presenting a defect in their capacity to trap iodide.

In this study, we successfully cloned human NIS gene and then transduced into B16 cells. New cell line (B16-A), which stably expressed hNIS, was established. Both studies in vitro and in vivo showed enhanced iodide uptake by B16-A, which suggests that NIS gene transfer can restore or newly induce radioiodide uptake in melanoma cells. It seems that the NIS has the potential to expand the role of nuclear medicine in the future, just as it has served as the base for the development of nuclear medicine in the past.

As is known that the efficacy of therapy depends not only on the amount of ^{131}I that accumulates in the tumor but also on the time it retains there;[12] viz. the biologic $T_{1/2}$ should match the physiologic $T_{1/2}$ of ^{131}I (8.04 d). In this study, the efflux of iodide were rapid both in vitro and in vivo, suggesting that ideal therapeutic effect may not be achieved by transfer NIS gene only. Further investigation is necessary to determine a method of maintaining more radioiodide in the cells long enough to produce greater therapeutic effects.

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References

1 Smanik PA, Liu Q, Furminger TL *et al*. BiochemBiophys Res Commun, 1996, **226**: 339-345

2 Shimura H, Haraguchi K, Miyazaki A *et al*. Endocrinology, 1997, **138**: 4493-4496

3 Nakamato Y, Saga T, Misaki T *et al*. J Nucl Med, 2000, **41**: 1998-1904

4 Haberkorn U, Henze M, Altmann A *et al*. J Nucl Med, 2001, **42**: 317-325

5 Spitzweg C, O'Connor MK, Bergert ER *et al*. Cancer Res, 2000, **60**: 6526-6530

6 Boland A, Ricard M, Opolon P *et al*. Cancer Res, 2000, **60**: 3484-3492

7 Mandell RB, Mandell LZ, Link CJ. Cancer Res, 1999, **59**: 661-668

8 Nakamato Y, Saga T, Misaki T *et al*. J Nucl Med, 2000, **41**: 1898-1904

9 Spitzweg C, Zhang S, Bergert ER *et al*. Cancer Res, 1999, **59**: 2136-2141

10 Spitzweg C, O'Connor MK, Bergert ER *et al*. Cancer Res, 2000, **60**: 6526-6530

11 Weiss SJ, Philp NJ, Grollman EF. Endocrinology, 1984, **114**: 1090-1098

12 Maxon HR Ⅲ, Thomas SR, Hertzberg VS *et al*. N Engl J Med, 1983, **309**: 937-941