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Original Article

Improved radioiodine-131 imaging of prostatic carcinoma using the sodium iodide symporter gene under control of the survivin promoter

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Abstract: Improvement of radioiodine accumulation in non-thyroidal tumors by transfecting the sodium iodide symporter (NIS) gene has been successfully investigated in many studies. However, regarding the uncertain iodine influx and efflux efficiencies in different cells, the optimal imaging time by radioiodine following NIS gene transport remains unclear. This study aimed to investigate the serial expression of NIS under control of survivin promoter in prostate cancer PC-3 cells and xenografts by adenoviral vector (Ad-Sur-NIS), and determine the optimal imaging time for radioiodine application. In vitro, the ^{125}I accumulation in Ad-Sur-NIS-infected PC-3 cells was 44 times higher than that in control cells ($P<0.05$). Moreover, the expression efficiency of NIS reached a peak at 48 h post transfection, at which a 1.9-fold or 1.4-fold increase of ^{125}I accumulation was found compared with 24 h or 72 h. In the clonogenic assay, the cell inhibition rates induced by ^{131}I were 93.4 ± 11.2% in Ad-Sur-NIS and 71.8 ± 10.1% in Ad-NIS infected cells, both of which were significantly higher than that in Ad-Sur-GFP infected cells (10.9 ± 1.9%, $P<0.05$). In in vivo studies, the ^{131}I uptake of tumor-to-muscle ratios were more prominent on day 2 (15.23 ± 4.55) and day-9 (9.78 ± 2.34) compared to the day 16 (1.29 ± 0.49), which showed a gradual reduction ($P<0.05$). Therefore, the Ad-Sur-NIS transfection allowed PC-3 tumor imaging by iodine-131 with an optimal time no later than 9 days post-transfection.

Keywords: Prostate carcinoma, sodium iodide symporter, survivin, radioiodine imaging

Introduction

The sodium iodide symporter (NIS) gene is expressed as a glycoprotein on the surface of thyroid follicular cells and mediates uptake and concentration of iodide [1, 2]. Preclinical studies [3-6], including our own [7-10], have shown that the gene transfer of NIS into various non-thyroidal cancer cells stimulates significant radioiodine uptake in vitro and in vivo.

Survivin, a member of the family of inhibitor of apoptosis proteins, has been reported to have fantastic efficiency of transcriptional activation [11-13]. Therefore, survivin-dependent transcriptional activation is a very useful means of tumor-specific gene expression for tumor-targeted gene imaging and therapy [14-16]. We have previously reported the high efficacy of radioiodine treatment by a plasmid vector expressing the NIS gene driven by the survivin promoter (Ad-Sur-NIS) in liver cancer cells and non-small cell lung cancer cells [7, 8]. However, with a lack of iodide transport vesicles, the efflux of radioiodine in these transfected cancer cells was unknown and thus, the optimal time of radioiodine image following Ad-Sur-NIS gene transfer remains unclear. In this study, we transfected the Ad-Sur-NIS gene under the control of survivin promoter into prostate cancer PC-3 cells, and investigated the in vitro radioiodine uptake, and evaluated the in vivo optimal time of radioiodine imaging.

Materials and methods

Cell lines

The human prostate cancer cell line PC-3 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were maintained in RPMI 1640 medium supplemented 10% calf serum (Gibco, Carlsbad, CA, USA), 100 IU/ml penicillin, and 100 ng/ml

streptomycin. Cells were grown at 37°C in an atmosphere of room air with 5% CO_2.

Production of recombinant adenovirus and cell infection

The recombinant adenovirus Ad-Sur-NIS, which uses the survivin promoter to drive NIS expression, was used as previously described [7, 8]. The recombinant adenovirus Ad-NIS, which uses no promoter to drive NIS expression, was used as previously described [7, 8]. The recombinant adenovirus Ad-Sur-GFP, which uses the survivin promoter to drive GFP expression, was used as a negative control [8, 9]. The PC-3 cells were added into 6-well plates with density of 1 × 10^6 cells per well, and then incubated for 24 h in RPMI 1640 medium prior to assay. The cells were then infected with 100 multiplicities of infection (MOI) Ad-Sur-NIS, Ad-NIS or Ad-Sur-GFP. After 2 h infection, the media were replaced with fresh culture media, and virus-infected cells were further maintained.

In vitro ^{125}I uptake experiments

After 24 h, 48 h and 72 h infection, 3.7 kBq of ^{125}I in 1 ml of medium without serum was applied to each well, respectively. After 30 min incubation, the cells were washed in cold PBS 3 times and detached with 0.5 ml trypsin; the radioactivity was measured using a γ-counter (No. 262 Nuclear Instrument Factory, Xi'an, China).

In vitro clonogenic assay

The procedure was performed as previously described [8, 9]. In brief, the PC-3 cells transfected with Ad-Sur-NIS, Ad-NIS or Ad-Sur-GFP were incubated in RPMI-1640 medium containing 370 kBq/ml ^{131}I for 7 h. After incubation, cells were then seeded onto six-well plates at a density of 1000 cells per well. After 1 week, colonies containing more than 30 cells were counted. All experiments were performed in triplicate. Results were expressed as the percentage of inhibited cells.

Immunohistochemical analysis of NIS protein expression

Animal experiments were reviewed and approved by the Sichuan University Animal Care and Use Committee. Six-week-old BALB/c nude mice were subcutaneously injected for xenograft tumor modeling with 1 × 10^7 PC-3 cells per mouse. The experiments lasted until the tumors achieved a diameter of 5 mm. The Ad-Sur-NIS (1 × 10^9 PFU), Ad-NIS (1 × 10^9 PFU) or Ad-Sur-GFP (1 × 10^9 PFU) were injected intratumorally by group (n = 3 for each group) for gene transfection in tumors.

To detect the NIS expression, resected tumors from nude mice were fixed in 4% paraformaldehyde for routine histopathological examination with immunohistochemical (IHC) examination with anti-NIS monoclonal antibody (Novus, Littleton, USA) [8, 9]. Each slide was evaluated using light microscopy and the staining was scored semi-quantitatively by assessing the intensity (on a 1-4 scale) and by estimating the percentage of positive cytoplasmic or membranous staining cells (on a 1-4 scale: 1, 1-25% staining; 2, 26-50% staining; 3, 51-75% staining; or 4, > 75% staining). With respect to both intensity and frequency, overexpression was defined as NIS positive tumors with diffuse cytoplasmic staining of moderate/strong intensity (≥ 25% cells and intensity score ≥ 2) in this study.

In vivo scintigraphic images

To determinate the optimal time of radionuclide diagnosis, the mice underwent radionuclide planar images on 2, 9, and 16 days after Ad-Sur-NIS injection. A single dose of 7.4 MBq ^{131}I was administered by intravenous injection to the mice, and 2 h post injection, the mice were anesthetized with 2% isoflurane and scanned with a γ camera (Philips Medical Systems, Milpitas, CA) equipped with a pinhole collimator. The 5 min static images were acquired with a 256 × 256 matrix.

Biodistribution of ^{131}I in the tumor-bearing mice

On days 2, 9, and 16 post Ad-Sur-NIS transfection, radioiodine uptake in tumor and muscle were assessed. A single dose of 370 kBq ^{131}I was injected into mice via tail vein, and the animals were sacrificed to collect tissues at 2 h post-injection. The tumor and muscle were dissected, weighed, and counted for radioactivity. The results were expressed as the tumor-to-muscle (T/M) ratio.

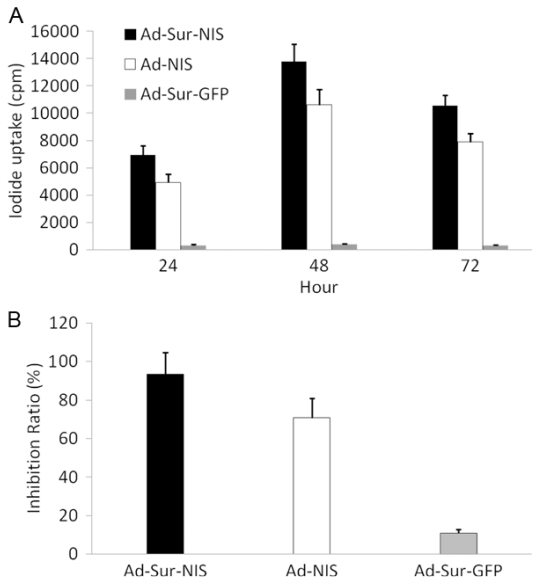

Figure 1. In vitro experiments. A. PC-3 cells were infected with 100 MOI Ad-Sur-NIS, Ad-NIS, or Ad-Sur-GFP, and ^{125}I uptake was measured on 24 h, 48 h, or 72 h. The maximal uptake was observed at 48 h following infection with Ad-Sur-NIS, and the uptake activity was 1.9 or 1.4 times higher than infection at 24 h or 72 h. PC-3 cells infected with Ad-Sur-NIS showed a 44-fold increase in ^{125}I accumulation compared with Ad-Sur-GFP (P<0.05). B. In vitro clonogenic assay. The inhibition rates induced by ^{131}I were 93.4 ± 11.2% in Ad-Sur-NIS and 71.8 ± 10.1% in Ad-NIS infected cells, both of which was significantly higher than that in Ad-Sur-GFP infected cells (10.9 ± 1.9%, P<0.05).

Statistical analysis

All data were expressed as mean ± standard deviation. For the in vitro cell and in vivo experiments, statistical significance was determined by Student's t-test, and statistical significance was achieved when the *P* value was <0.05.

Results

In vitro ^{125}I uptake assays

The ^{125}I uptake assays were performed to confirm the efficiency of transfection with 100 MOI Ad-Sur-NIS, Ad-NIS or Ad-Sur-GFP at 24 h, 48 h and 72 h in PC-3 cells post transfection. The peak cellular uptake of ^{125}I was observed at 48 h post transfection of Ad-Sur-NIS , in which the ^{125}I accumulation was 44 times higher than that of control Ad-Sur-GFP-infected cells (P<0.05) (**Figure 1A**). Moreover, 48 h displays indicated 1.9-fold higher than 24 h and 1.4-fold higher than 72 h, indicating the optimal transfection time should be 48 h for PC-3 cancer cells (**Figure 1A**).

Clonogenic assay of PC-3 cells

Cell viability was assessed for evaluation of the radioiodine sensitivity in these trans-gene cells by incubating cells with infected with 370 kBq ^{131}I for 7 h. The inhibition rates induced by ^{131}I were 93.4 ± 11.2% in Ad-Sur-NIS and 71.8 ± 10.1% in Ad-NIS infected cells, both of which were significantly higher than that in Ad-Sur-GFP infected cells (10.9 ± 1.9%, P<0.05) (**Figure 1B**). These results demonstrated coupling Ad-Sur-NIS infection and ^{131}I treatment specifically and efficiently led to PC-3 cell death in vitro, indicating potential for iodine-131 diagnosis and radiotherapy.

Immunohistochemical staining of NIS expression

In tumor tissues with transfection of Ad-Sur-NIS, immunohistochemical results revealed the typical NIS positive signals manifested by diffuse cytoplasmic staining (scale 4) (**Figure 2A**). The tumor infected with Ad-NIS was evaluated as scale 3 (**Figure 2B**). In contrast, the tumor infected with Ad-Sur-GFP was negative (1 scale) (**Figure 2C**).

The optimal time of ^{131}I scintigraphic imaging and biodistribution studies

To determine the optimal time of radionuclide imaging, on 2, 9 and 16 days post Ad-Sur-NIS injection, the gene-transfected mice underwent planar imaging at 2 h post ^{131}I administration. SPECT images indicated clearest visualization of thyroid, stomach, and tumor at 2 days post gene transfection (**Figure 3A**). Excess radioiodine was excreted through the bladder and the high uptake of ^{131}I was gradually cleared from the thyroid in the following days. Then, the signal of ^{131}I was gradually reduced throughout the whole body thereafter while the tumor and stomach were still readily visualized at 9 days post gene transfection (**Figure 3B**). By day 16 (**Figure 3C**), a residual low signal of ^{131}I could be obtained in stomach and bladder, while the tumor showed marginal ^{131}I uptake, indicating the Ad-Sur-NIS loss from PC-3 tumor.

Biodistribution data indicated a statistically significant tumor-to-muscle (T/M) ratio of 15.23 ±

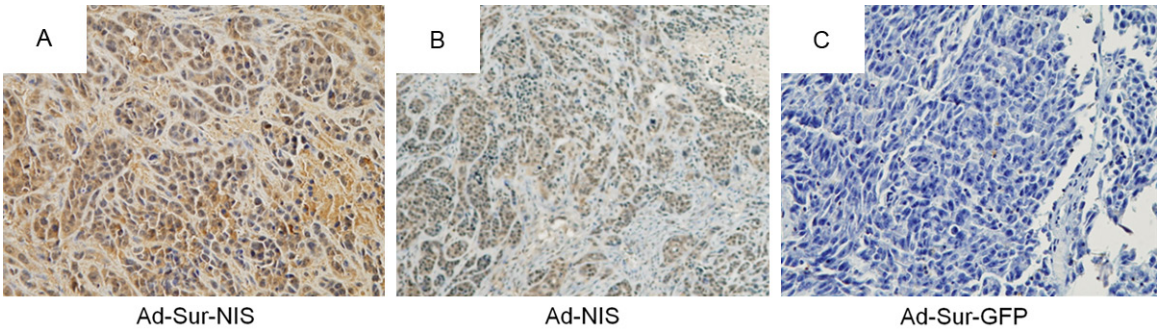

Figure 2. Immunohistochemical staining of NIS expression (× 200). The tumors infected with Ad-Sur-NIS, Ad-NIS and Ad-Sur-GFP were evaluated as 4 scale (A), 3 scale (B) and 1 scale (C).

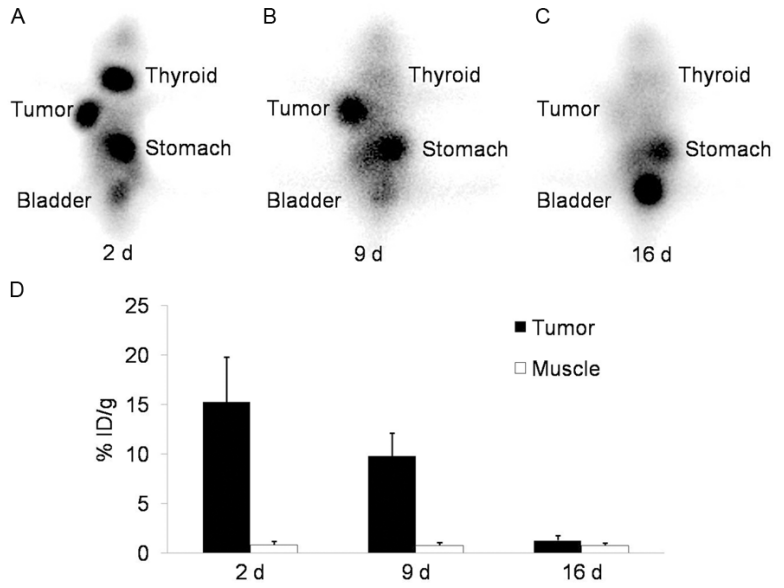

Figure 3. The optimal time of ^{131}I scintigraphic imaging and biodistribution studies. Serial images of PC-3 tumors bearing mouse day 2, 9, 16 after Ad-Sur-NIS injection. The tumors were readily visualized on day 2 (A) and day 9 (B), but marginal ^{131}I uptake was seen on day 16 (C). The images showed decreasing ^{131}I uptake over time, reflecting decreased NIS gene expression. (D) The ^{131}I uptake of T/M ratios on days 2, 9, 16 after Ad-Sur-NIS injection were 15.23 ± 4.55, 9.78 ± 2.34 and 1.29 ± 0.49, respectively ($P<0.05$). The T/M ratio at day 2 after Ad-Sur-NIS injection was the highest of the other time points.

4.55 at day 2 and 9.78 ± 2.34 at day 9; then the radioiodine decreased along with the metabolic deduction, and the T/M ratio declined to 1.29 ± 0.49 at day 16 (**Figure 3D**). Therefore, the optimal time of ^{131}I imaging should be no later than 9 days after infection with Ad-Sur-NIS in PC-3 tumors.

Discussion

Many studies have demonstrated the possibility of radioiodine imaging or therapy by transporting the NIS gene into iodine-free cancer cells [17-19]. Furthermore, this transgene efficiency could even be improved by serially transfecting NIS following a proper promoter, such as survivin [16, 20]. In previous studies, our group had reported successful Ad-Sur-NIS expression in liver cancer HepG2 cells and non-small cell lung cancer A549 cells with this strategy [7, 8]. However, the optimal timing of radioiodine application was still unclear since most past research was performed at only a single time point. Therefore, the present study aimed to determine the optimal time of radioiodine serial imaging following Ad-Sur-NIS transfer into prostate cancer PC-3 cells.

In terms of ensuring tumor-specific radiation exposure, the application of tumor-specific survivin promoters offers the ability to induce NIS-selective expression in PC-3 cells. Radionuclide uptake and inhibition rate of Ad-Sur-NIS were higher than those of Ad-NIS in PC-3 cells ($P<0.05$). On the scale of IHC staining experiment, the PC-3 tumors infected with Ad-Sur-NIS were higher than those with Ad-NIS. These data demonstrated that the survivin promoter was able to induce tumor-specific functional NIS expression in PC-3 cells.

Consistent with our observation, PC-3 cells infected with Ad-Sur-NIS were able to concentrate radioactive iodine-125 specifically and effectively, and 48 h infection of Ad-Sur-NIS indicated the best trans-gene efficiency with the maximum ^{125}I uptake. The ^{125}I accumulation in Ad-Sur-NIS-infected cells was 44 times higher than in Ad-Sur-GFP-infected cells ($P<0.001$). Clonogenic assays demonstrated strong inhibition of clone formation of Ad-Sur-NIS-infected cells by ^{131}I, confirming that ^{131}I uptake was essentially dependent on NIS expression. In an in vivo study, we successfully transferred Ad-Sur-NIS into PC-3 tumors, and serially measured transferred Ad-Sur-NIS gene expression 2, 9 and 16 days by scintigraphic imaging and biodistribution. These results showed that 2-9 days post-intratumoral injection was an optimal time for radioiodine imaging using the Ad-Sur-NIS gene.

In conclusion, radioiodine uptake was successfully increased in PC-3 tumors by Ad-Sur-NIS gene transfer in vitro and in vivo. The optimal time for radioiodine administration may be 2-9 days after Ad-Sur-NIS transfer into PC-3 tumors.

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Disclosure of conflict of interest

None.

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