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Original Article

Targeting of pertechnetate imaging of HepG2 hepatocellular carcinoma through the transduction of the survivin promoter controls the sodium iodide symporter

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Abstract: Currently, the intratumoral (i.t.) injection of adenovirus-mediated cancer gene therapy has been reported in numerous investigations. However, the intravenous (i.v.) injection of adenovirus is more suited for disseminated tumors and distant metastatic lesions. The survivin promoter, which has tumor-selective capability, was used to construct an adenoviral vector, and its feasibility to carry the sodium iodide symporter (NIS) gene for potential tumor-targeting transfection into carcinoma was evaluated in this study. An in vitro cellular assay using HepG2 hepatocellular carcinoma (HCC) cells exhibited iodide uptake after infection with Ad-Sur-NIS, and the uptake reached a maximum level at 30 min. The effective half-life of ^{125}I efflux from Ad-Sur-NIS infected HepG2 cells was 16.67 ± 1.08 min. Moreover, in vivo transfection of Ad-Sur-NIS via i.v. injection induced more effective transfection and, accordingly, induced higher uptake of pertechnetate in HCC xenograft tumors than did the non-specific transfection of Ad-CMV-NIS (i.v.) ($P<0.05$), indicating possible selective NIS gene-transfecting image strategies by the survivin promoter.

Keywords: Hepatocellular carcinoma, sodium iodide symporter, survivin promoter, intravenous injection, pertechnetate imaging

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers in East Asians, and its incidence is also increasing in the Western world [1]. However, advanced HCC has a poor prognosis despite various therapeutic options [2]. Partial hepatectomy or liver transplantation, chemotherapy, radiation therapy, or a combined modality approach is also only partially effective against advanced HCC [3, 4]. Therefore, the development of new therapeutic strategies is indispensable. Gene therapy, as a promising method, may be a potential future treatment strategy for HCC [5, 6].

The sodium iodide symporter (NIS) is an intrinsic membrane glycoprotein that can transport iodide into thyroid follicular cells [7, 8]. NIS is the molecular basis for the diagnosis and therapeutic management of thyroid diseases, including differentiated thyroid cancer [9, 10]. Many investigations, including our own, have shown that the gene transfer of NIS into various non-thyroidal cancer cells confers increased radioiodine uptake [11-15]. Therefore, NIS is the potential gene for the molecular imaging and targeted therapy of non-thyroidal tumors.

In terms of ensuring tumor-specific radiation exposure, the application of tumor-specific promoters can drive NIS selective expression in tumor cells. Survivin, the smallest member of the inhibitor of apoptosis protein family, is highly expressed in most human cancers but is undetectable in normal adult tissues [16, 17]. Our previous work in lung cancer cells and prostate cancer cells also demonstrated the high efficacy of radioiodine therapy after tumor-selective Ad-Sur-NIS gene delivery via intratumoral (i.t.) injection [11, 12]. Compared with the i.t. injection of Ad-Sur-NIS, the intravenous (i.v.) injection of Ad-Sur-NIS will be more suitable for disseminated tumors and distant metastatic

lesions [15, 18]. Therefore, the i.v. injection of Ad-Sur-NIS could be useful for expanding this strategy in clinical application.

In this study, we aimed to investigate the i.v. injection of a gene for in vivo biodistribution and scintigraphy experiments of mice bearing HepG2 cells by delivery of an adenovirus vector containing the NIS gene under the control of the survivin promoter.

Materials and methods

Cell lines

The human HCC cell line HepG2 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in RPMI 1640 medium containing 10% calf serum (Gibco, Carlsbad, CA, USA), 100 IU/ml penicillin, and 100 ng/ml streptomycin at 37°C in a humidified 5% CO_2 atmosphere.

Construction of recombinant adenovirus

The recombinant adenovirus Ad-Sur-NIS, which uses the survivin promoter to drive NIS expression, was used as previously described [12]. The recombinant adenovirus Ad-CMV-NIS, which uses the cytomegalovirus (CMV) promoter to drive NIS expression, was used as a positive control [12]. The recombinant adenoviruses Ad-Sur-GFP and Ad-CMV-GFP were used as negative controls [11, 12].

In vitro ^{125}I uptake and efflux experiments

The HepG2 cells were plated in 6-well plates and were cultured with RPMI 1640 medium. When the cells reached confluence (approximately 1×10^6 cells per well), they were incubated at 100 multiplicity of infection (MOI) per well of Ad-Sur-NIS, Ad-CMV-NIS, Ad-Sur-GFP or Ad-CMV-GFP for at 37°C and 5% CO_2. After 2 h, the media were replaced with fresh culture media, and virus-infected cells were further maintained. After 48 h, the cells were quickly washed with 1 ml of phosphate-buffered saline (PBS) and were incubated with 3.7 kBq ^{125}I in 1 ml of medium without serum. To evaluate the time course of the iodide uptake, the cells were incubated for 5, 10, 15, 20, 30, 60 and 90 min in the ^{125}I solution. The cells were then washed with 1 ml of ice-cold PBS, and the radioactivity of the detached cells was counted using a γ-counter (No. 262 Nuclear Instrument Factory, Xi'an, China).

To determine the ^{125}I efflux, the cells were incubated with 3.7 kBq ^{125}I in 1 ml of medium without serum for 30 min. The cells were washed twice with PBS, followed by the addition of medium without serum and further incubation. At specified time points (5, 10, 15, 20, 25 and 30 min), the buffer was removed, and its radioactivity was measured. After the last time point, the cells were collected and immediately lysed to determine the residual radioactivity.

In vivo imaging acquisition

The experiments involving animals were performed with the approval of the Sichuan University Animal Care and Use Committee. Ten million (1×10^7) HepG2 cells were transplanted subcutaneously into the flank region of 6-week-old BALB/c nude mice. The nude mice weighed 18-20 g. The experiments started when tumors had reached 8-10 mm in diameter. Two days after the i.v. injection of 1×10^9 PFU Ad-Sur-NIS and Ad-CMV-NIS, the mice were anesthetized with 2% isoflurane and were injected with 18.5 MBq $^{99m}TcO_4^-$. At 2 h post injection, the mice were scanned with a gamma camera equipped with a low-energy, high-resolution pinhole collimator (Philips Medical Systems, Milpitas, CA). Each image was acquired with a 256×256 matrix and was magnified two times.

Immunohistochemical staining of NIS protein expression

To detect the NIS expression, resected tumors from nude mice were fixed in 4% paraformaldehyde for 24 h. The samples were immunostained using a standard streptavidin-biotin labeling protocol as described previously [12].

Biodistribution of ^{125}I in the tumor-bearing mice

At 2 days after adenovirus treatment, radioiodine uptake in organs were assessed. The dose of 370 kBq ^{125}I was injected into mice, and the animals were sacrificed at 2 h post-injection, and the tumor, liver and muscle were dissected, weighed and counted for radioactivity. The results were expressed as the percentage of the injected dose per gram of tissue.

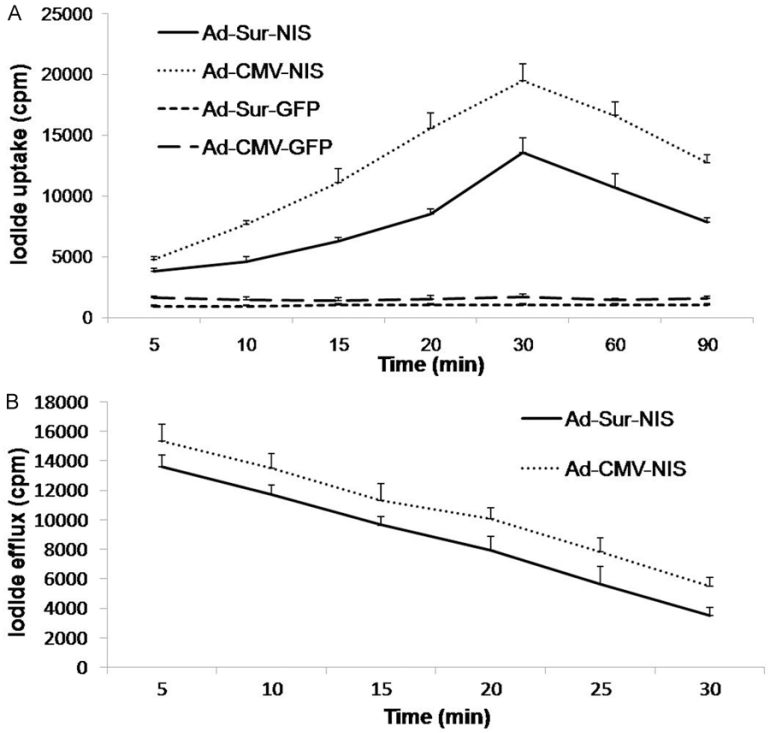

Figure 1. In vitro experiments. A. Ad-Sur-NIS- and Ad-CMV-NIS-infected HepG2 cells showed significant ^{125}I uptake ability compared with Ad-Sur-GFP- and Ad-CMV-GFP-infected controls (P<0.001). Iodide uptake increased within 30 min due to the incubation of the Ad-Sur-NIS- and Ad-CMV-NIS-infected HepG2 cells at different times with 3.7 kBq of ^{125}I. B. The ^{125}I efflux rates were similar in HepG2 cells infected with Ad-Sur-NIS and Ad-CMV-NIS ($T_{1/2}$ = 16.67 ± 1.08 min, and $T_{1/2}$ = 18.34 ± 1.59 min, P>0.05).

Statistical analysis

The data were expressed as mean ± standard derivation. In vitro cell and vivo biodistribution experiments, statistical significance was determined using Student's t-test, and statistical significance was achieved when the *P* value was <0.05.

Results

^{125}I uptake and efflux studies in vitro

To test the transduction conditions in Ad-Sur-NIS-, Ad-CMV-NIS-, Ad-Sur-GFP- and Ad-CMV-GFP-infected HepG2 cells, functional assays quantifying ^{125}I uptake were performed. As shown in **Figure 1A**, the ^{125}I uptake was rapidly increased and reached a maximum level at 30 min in HepG2 cells infected with Ad-Sur-NIS and Ad-CMV-NIS. HepG2 cells infected with Ad-Sur-NIS and Ad-CMV-NIS exhibited significant ^{125}I uptake, with the values of 13584 ± 1197 c.p.m. and 19496 ± 1360 c.p.m. By contrast, merely ^{125}I uptake above the background level was observed in HepG2 cells infected with Ad-Sur-GFP and Ad-CMV-GFP, with the values of 326 ± 49 c.p.m. and 447 ± 53 c.p.m. Ad-Sur-NIS- and Ad-CMV-NIS-infected HepG2 cells showed significant ^{125}I uptake ability compared with Ad-Sur-GFP- and Ad-CMV-GFP-infected controls (P< 0.001). The uptake experiment indicated that ^{125}I uptake was essentially dependent of NIS expression. **Figure 1B** showed the amount of ^{125}I present within the cells as a function of time. The effluxes of ^{125}I from Ad-Sur-NIS- and Ad-CMV-NIS-infected HepG2 cells were similar, with effective half-life ($T_{1/2}$) values of 16.67 ± 1.08 min and 18.34 ± 1.59 min, respectively (P>0.05). The efflux experiment indicated that the $T_{1/2}$ of Ad-Sur-NIS- or Ad-CMV-NIS-infected HepG2 cells were similar.

Scintigraphic imaging of tumor-bearing mice in vivo

To evaluate the visualization effect via adenovirus infection in tumors, scintigraphy was performed on tumor-bearing mice with $^{99m}TcO_4^-$ pertechnetate. Normal physiological uptake values were observed in the thyroid, stomach and bladder. The tumors infected with i.v. injection of Ad-Sur-NIS were visible, and only background activity was observed in the normal liver tissue (**Figure 2A**). The tumors infected with Ad-CMV-NIS i.v. were not visible, but the normal liver tissue exhibited significant uptake of $^{99m}TcO_4^-$ (**Figure 2B**).

Immunohistochemical staining results of NIS expression in the HepG2 xenografts

The tumors infected with Ad-Sur-NIS i.v. exhibited NIS-specific immunoreactivity (**Figure 2C**). By contrast, the tumors infected with

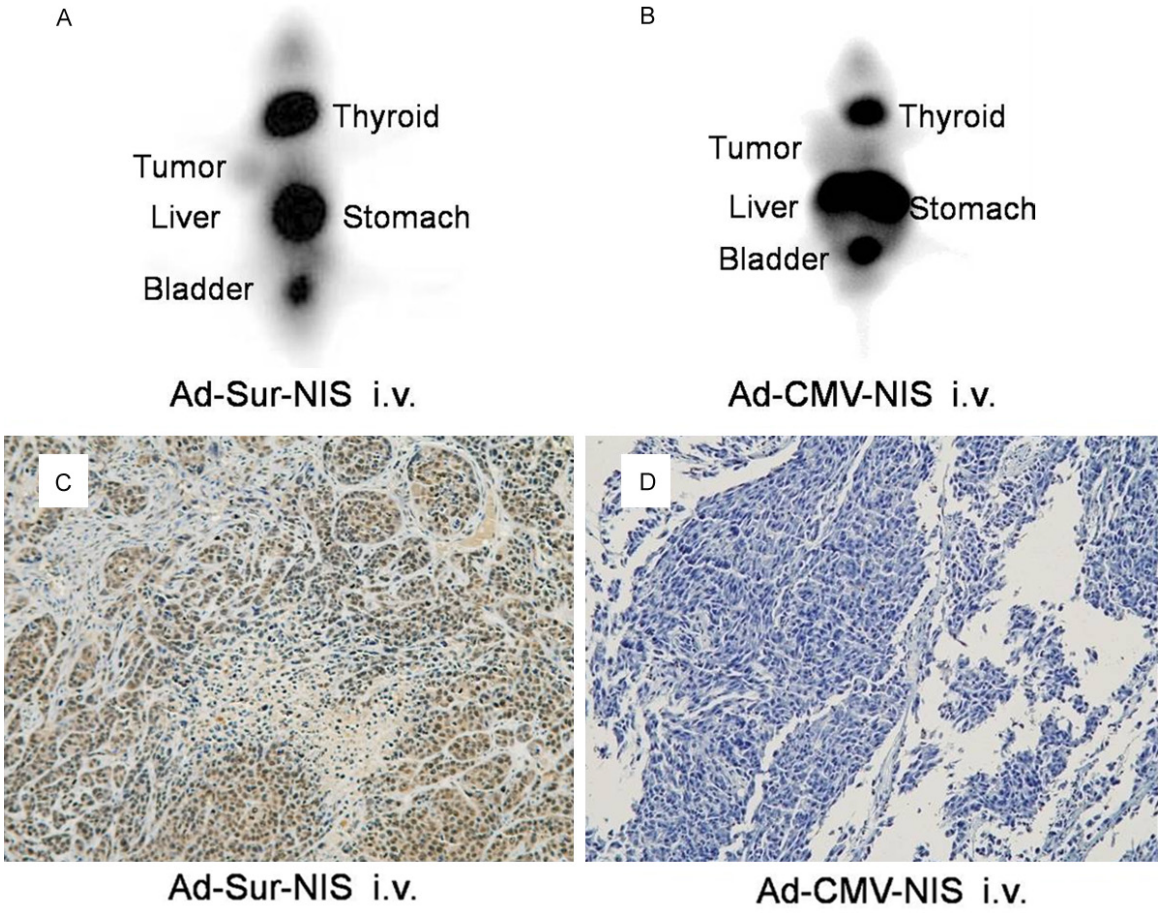

Figure 2. $^{99m}TcO_4^-$ pertechnetate scintigraphic images and immunohistochemical staining results (×200). A. The tumors infected with the i.v. injection of Ad-Sur-NIS were visible, and only the background activity was observed in the normal liver tissue. B. The tumors infected with the i.v. injection of Ad-CMV-NIS were not visible, but the normal liver tissue exhibited a significant uptake of $^{99m}TcO_4^-$. C. The tumors infected with the i.v. injection of Ad-Sur-NIS exhibited NIS-specific immunoreactivity. D. By contrast, the tumors infected with the i.v. injection of Ad-CMV-NIS did not exhibit NIS-specific immunoreactivity.

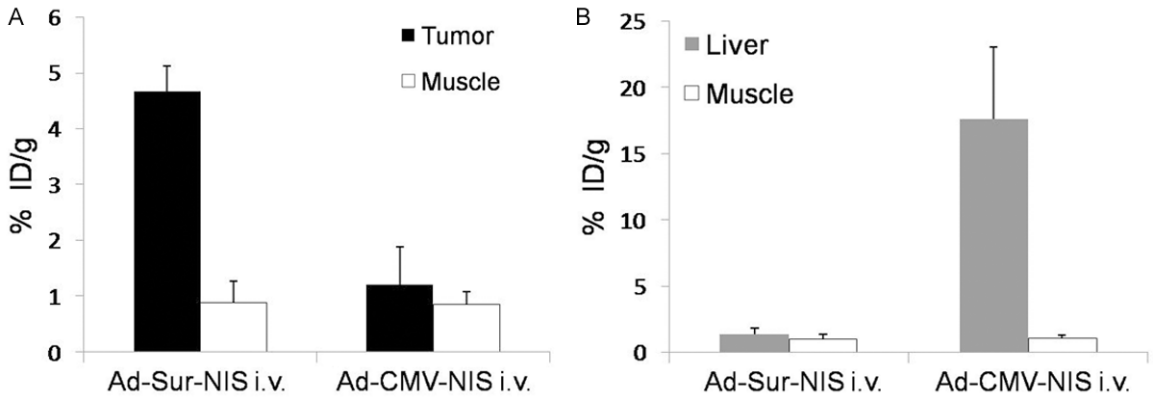

Figure 3. Biodistribution of ^{125}I in mice with Ad-Sur-NIS- and Ad-CMV-NIS-injected HepG2 xenografts. A. The ^{125}I accumulated in tumors via the i.v. injection of Ad-Sur-NIS was higher than that via the i.v. injection of Ad-CMV-NIS (4.66 ± 0.45 vs 1.21 ± 0.66, $P<0.05$). B. The ^{125}I accumulated in the liver via the i.v. injection of Ad-Sur-NIS was lower than that via the i.v. injection of Ad-CMV-NIS (1.38 ± 0.45 vs 17.59 ± 5.48, $P<0.05$).

Ad-CMV-NIS i.v. did not exhibit NIS-specific immunoreactivity (**Figure 2D**).

^{125}I biodistribution studies in tumor-bearing mice

The biodistribution values of ^{125}I in mouse tissues are provided in **Figure 3**. The ^{125}I accumulated in tumors via i.v. injection of Ad-Sur-NIS was higher than that via i.v. injection of Ad-CMV-NIS (4.66 ± 0.45 vs 1.21 ± 0.66, P<0.05). The ^{125}I accumulated in the liver via the i.v. injection of Ad-Sur-NIS was lower than that via the i.v. injection of Ad-CMV-NIS (1.38 ± 0.45 vs 17.59 ± 5.48, respectively; P<0.05). The tumor specificity of Ad-Sur-NIS was confirmed via biodistribution, which exhibited selective uptake of ^{125}I in tumors and a lack of ^{125}I uptake in the liver.

Discussion

The cloning and characterization of the NIS gene have paved the way for radionuclide imaging and therapy in non-thyroidal cancer [19, 20]. Tissue-specific promoters may serve as targeting mechanisms for gene expression and have been described to mediate tissue-specific expression. In the context of the NIS gene therapy of different types of cancer, the promoters could introduce cancer specificity for improved safety and efficacy [21-23]. In previous studies, we have reported successful NIS expression via the application of the survivin promoter in A549 non-small cell lung cancer cells and PC3 prostate cancer cells following i.t. injection [11, 12]. Although our previous data indicated a potential strategy for the imaging and therapy of iodine-insensitive tumors by NIS gene transfection, there remains a barrier before clinical application in selectively transferring the NIS gene into tumors. The i.t. drug injection is acceptable in notably few cases, while the i.v. drug injection requires a comparably effective but more selective promoter to mediate targeted and efficient NIS gene transfer with the potential to reach tumor metastases.

This functional NIS protein expression after adenoviral NIS gene transfer into HepG2 cells was confirmed by measurement of the in vitro ^{125}I uptake. Ad-Sur-NIS- and Ad-CMV-NIS-infected HepG2 cells showed significant ^{125}I uptake ability compared with Ad-Sur-GFP- and Ad-CMV-GFP-infected controls (P<0.001). The uptake experiment indicated that ^{125}I uptake was essentially dependent on NIS expression. The effluxes of ^{125}I from Ad-Sur-NIS- and Ad-CMV-NIS-infected HepG2 cells were similar (P>0.05), although CMV was a strong promoter. The effluxes of time were rapid, possibly because of the lack of iodide organification and the lack of an increase in the retention time of accumulated radioiodine in NIS gene-infected HepG2 cells [24].

The radionuclide uptake of i.v. injection of Ad-Sur-NIS was higher than that of i.v. injection of Ad-CMV-NIS in tumors (P<0.05) but lower than that of i.v. injection of Ad-CMV-NIS in the liver (P<0.05). Therefore, the i.v. injection of Ad-Sur-NIS could enhance radionuclide accumulation in hepatoma xenografts based on the tumor specificity of the survivin promoter, which would be more suitable for disseminated tumors and distant metastatic lesions. The CMV promoter showed no tumor specificity, and the normal liver tissues exhibited significant iodide uptake via the i.v. injection of Ad-CMV-NIS, and the results were similar to other reports [19, 25]. The livers of mice accumulated high levels of technetium after the i.v. injection of Ad-CMV-NIS as shown by γ-camera imaging, resulting in markedly reduced tumoral transduction as seen by almost no technetium uptake activity of HCC xenografts.

In conclusion, the i.v. injection of Ad-Sur-NIS could help to verify the applicability of targeted NIS gene imaging for hepatoma xenografts. The i.v. injection of Ad-Sur-NIS will be more suitable for disseminated tumors and distant metastatic lesions in clinical application.

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Disclosure of conflict of interest

None.

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