Rat Sodium Iodide Symporter for Radioiodide Therapy of Cancer

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ABSTRACT
Design and development of new approaches for targeted radiotherapy of cancer and improvement of therapeutic index by more local radiation therapy are very important issues. Adenovirus-mediated delivery of the sodium iodide symporter (NIS) gene to cancer cells is a powerful technique to concentrate lethal radiation in tumor cells and eradicate tumors with increased therapeutic index. A replication-defective adenoviral vector expressing the rat NIS gene (Ad-rNIS) was used for in vitro gene delivery and into human prostate cancer xenografts to study antitumor effect. Robust function of the rat symporter was detected in DU145, T47D, and HCT-15 human cancer cell lines transduced with Ad-rNIS. All three cancer cell lines successfully transferred functionally active rat symporter to the plasma membrane, resulting in very high levels of iodine-125 accumulation. Three-dimensional multicellular tumor spheroids derived from DU145 human prostate cancer cells were transduced with Ad-rNIS and incubated with 131I for 24 hours. After treatment, spheroids rapidly decreased in size and disappeared within 10 days. In vivo data revealed an inhibition of tumor growth in athymic nude mice after intratumoral Ad-rNIS injection followed by 131I administration. Eighty-eight percent of experimental mice survived >30 days, whereas control groups had only 18% survival >30 days. This is the first report that demonstrates the rat NIS gene can effectively induce growth arrest of human tumor xenografts after in vivo adenoviral gene delivery and 131I administration. The data confirm our hypothesis that the rat NIS gene is an attractive suicide gene candidate for cancer treatment.

INTRODUCTION
In the United States, ~550,000 cancer patients still die from progressive disease each year despite significant progress in both cancer diagnosis and therapy (1). Approximately 60% of all cancer patients receive radiation treatment as primary therapy or for palliation or as an adjunct to surgery and chemotherapy (2). Radiation therapy attempts to deliver a lethal dose of irradiation to a defined tumor site while causing minimal damage to surrounding healthy tissue. There is an optimal dose of radiation that gives the greatest therapeutic index, and tumor control curves diverge maximally from tissue damage curves. Unfortunately, the therapeutic index for external beam radiation therapy is very narrow, and often substantial damage to normal tissue occurs (2). Precise delivery of lethal radiation to tumor cells is most desirable for patients. Much scientific effort has been focused on design and development of new approaches for targeted radiotherapy of cancer and improvement of the therapeutic index by more local radiation therapy (3–11). Targeted delivery of the sodium iodide symporter (NIS) gene to cancer cells could be one approach to concentrate lethal radiation in tumor cells and eradicate tumors with an increased therapeutic index (12, 13).

NIS is a transmembrane glycoprotein found on the surface of thyroid cells, and its function results in a 20 to 40-fold increase in intracellular iodine concentration over plasma levels (14, 15). It has previously been shown that both viral and nonviral NIS gene transfer into nontumor origin cancer cells resulted in NIS gene expression and NIS-specific iodine accumulation within those cells (14, 16–18). Delivery of the NIS gene using adenoviral vectors results in significantly higher levels of iodine uptake in transduced cells when compared with retroviral or nonviral gene delivery (19, 20). Furthermore, significant tumor reduction was demonstrated after intratumoral adenoviral delivery of the human NIS gene to athymic nude mice followed by iodine-131 administration (21).

In our previous study, we directly compared the human and rat NIS gene for their abilities to concentrate radioiodine into human and rodent cancer cells in vitro. NIS-specific 125I uptake in multiple cell lines was demonstrated after transduction with retroviral vectors expressing either the human or rat NIS gene. Surprisingly, iodine uptake was consistently higher with the rat NIS gene (up to 5-fold greater) in comparison to the human gene when a variety of human tumor cell lines were tested (22). In this study, an adenoviral vector expressing the rat NIS (Ad-rNIS) gene was used for in vivo gene delivery into human prostate cancer xenografts. After treatment with Ad-rNIS, animals that received 131I demonstrated significant NIS-specific inhibition of tumor growth. Our results indicate a gene therapy approach using the rat NIS gene, and radioactive iodine may be an effective therapeutic option for cancer treatment.

MATERIALS AND METHODS

Cell Culture. DU145 (human prostate metastatic carcinoma), HCT-15 (human colorectal adenocarcinoma), and 293
(primary human embryonic kidney transformed by human adenovirus type 5 DNA) cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% fetal bovine serum and 2 mmol/L l-glutamine (D10; Life Technologies, Inc. Rockville, MD). T47D (human breast ductal carcinoma) cells (American Type Culture Collection) were grown in D10 supplemented with 1 mmol/L sodium pyruvate, 0.1 mmol/L nonessential amino acids, 10 mmol/L HEPE (pH 7.0; Life Technologies, Inc.), and 1 μg/mL insulin (Sigma, St. Louis, MO).

Construction of the Recombinant Adenovirus Containing the Rat NIS Gene. Ad-rNIS is a ΔE1-ΔE3 replication-defective recombinant adenovirus containing the rat NIS gene under control of the cytomegalovirus immediate early promoter. The rat NIS gene was cloned from pLNC-rNIS retroviral vector (12) into the pS5 adenoviral shuttle vector (23). pS5-rNIS was linearized with EcoRI and the Adeno-Quest kit (Qbiogene, Carlsbad, CA) was used to create recombinant adenovirus. Adenoviral clones containing the rNIS gene were selected by PCR using primers complimentary to both the 49 to 68- and 394 to 413-bp regions of the rNIS gene 5’-region: 5’-TACGGGGTGTGTCCG-GACCAT-3’ (sense) and 5’-AAGTACTGCAGCGTCCCGCA-3’ (antisense). Positive recombinant adenoviral clones were then analyzed individually for rNIS gene delivery to DU145 prostate cancer cells using an 125I uptake assay reflecting functional activity of the transporter. A recombinant adenoviral clone resulting in NIS activity in DU145 cancer cells was additionally purified twice by plaque assay using 293 cells. Recombinant adenovirus containing human NIS gene was constructed following the same pattern. Dr. Sissy Jhiang (The Ohio State University, Columbus, OH) kindly provided the full-length human NIS gene (22). As a control, a recombinant adenovirus (adenoviral vector containing herpes simplex virus thymidine kinase) containing the herpes simplex virus thymidine kinase gene was used (24). All recombinant adenoviruses were propagated in 293 cells and purified using conventional methods. Viral titers were determined by two methods: tissue culture infectious dose (TCID50) and plaque assay and expressed in plaque-forming units (PFU)/mL.

Anti-rNIS Antibody Production. Site-directed polyclonal anti-COOH-terminal NIS antibody was generated as reported by Levy et al. (25). A peptide spanning amino acids 600 to 618 of the rat NIS COOH-terminal region was synthesized by the Iowa State University Protein Facility (Ames, IA). The synthetic peptide was conjugated to keyhole limpet hemocyanin using the Imject Immunogen EDC kit with mcKLH (Pierce, Rockford, IL). Female New Zealand White rabbits were immunized with rNIS-peptide conjugate (Genemed Synthesis, Inc., San Francisco, CA). Sera from two rabbits were combined, and the IgG fraction was purified by affinity chromatography on protein A-Sepharose (Amersham Pharmacia Biotech, Piscataway, NJ) in one step by eluting with 0.1 mol/L sodium citrate (pH 3.5) and neutralizing with 1.0 mol/L Tris-HCl (pH 8.5).

Iodide Uptake Assay. Experiments were performed using a modified method of Weiss et al. (26). Briefly, DU145, T47D, and HCT-15 cells were seeded into 24-well tissue culture plates in D10. After 24 hours of incubation, cells were infected with Ad-rNIS. At 48 hours after infection, cells were washed with buffered Hank’s Balanced Salt Solution (Life Technologies, Inc.). Iodide uptake was initiated by adding 0.5 mL of buffered HBSS containing 0.057 to 0.345 nmol/L carrier-free Na125I (DuPont NEN, Boston, MA) and 5 to 300 μmol/L sodium iodide to give a specific activity of 88 to 880 nCi/mL. Cells were incubated (37°C, 5.0% CO2) for 30 minutes. For NIS-specific inhibition assays, sodium perchlorate (Sigma) was added to a final concentration of 30 μmol/L immediately after the addition of radioisotope. Reactions were rapidly terminated by washing the cells twice with ice-cold buffered HBSS, and cells were solubilized in 1% Triton X-100 (Sigma) for 20 minutes. The total radioactivity of accumulated iodide was measured using a gamma counter (Wallac, Turku, Finland). The number of cells per assay was determined by harvesting and counting cells in a control tissue culture plate.

Membrane Fraction Preparation. Membrane fractions were prepared from DU145, T47D, and HCT-15 cells as described previously (27, 28). Briefly, cells were seeded into 175-cm2 tissue culture flasks, transduced with Ad-rNIS for a predetermined period of time, washed with PBS, and harvested. Cells were homogenized in ice-cold buffer containing 250 mmol/L sucrose, 10 mmol/L HEPE (pH 7.5), 1 mmol/L EDTA, 10 μg/mL leupeptin, 10 μg/mL aprontin, and 1 mmol/L phenylmethylsulfonyl fluoride (Sigma). The homogenate was centrifuged twice at 500 × g for 10 minutes at 4°C to remove cell debris and organelles. The final centrifugation step at 100,000 × g was performed for 20 minutes, and the membrane pellet was resuspended in an appropriate volume of ice-cold buffer containing 250 mmol/L sucrose, 10 mmol/L HEPE (pH 7.5), and 1 mmol/L MgCl2. The protein concentration of membrane samples was determined by Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA).

Western Blot Analysis. Aliquots of membranes (20 to 30 μg) prepared from cancer cells were diluted with 5× loading buffer and heated at 37°C for 30 minutes (28), and membrane proteins were separated by SDS-PAGE. After gel electrophoresis for 1 hour, proteins were transferred to a nitrocellulose membrane using electroblotting. Nitrocellulose membranes were preincubated for 1 hour in buffer containing 20 mmol/L Tris (pH 7.5), 0.14 mol/L NaCl, 5% nonfat dried milk, and 0.05% Tween 20 to block nonspecific protein binding sites. Membranes were then incubated with primary rNIS-specific antibody overnight. After washing the membranes, they were incubated for 1 hour with a secondary antirabbit antibody conjugated with alkaline phosphatase (Sigma). 5-Bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium tablets (Sigma) dissolved in distilled water were used as a substrate for alkaline phosphatase to reveal rNIS-specific staining.

Immunocytochemical Detection of rNIS in DU 145 Cells. DU145 (2.5 × 105 cells per well) were plated into a 24-well plate, and after 24 hours, all cells were transduced with 50 multiplicity of infection (MOI) of Ad-rNIS. After 48 hours, the cells in the plate were washed and fixed with 4% paraformaldehyde in 1× PBS (pH 7.2) at 4°C for 30 minutes. Cells were washed and permeabilized for 30 minutes with buffer containing 0.3% Triton X-100 at room temperature because rNIS-specific antibody only recognizes the intracellular domain of the symporter. The cells were incubated with rabbit anti-NIS primary antibody or preimmune serum diluted in 1× PBS and 10% normal horse serum for 2 hours at room temperature. The cells were incubated subsequently with goat antirabbit secondary...
antibody conjugated with alkaline phosphatase at room temperature for 1 hour. Secondary antibody dilutions were made in 1× PBS supplemented with 10% normal horse serum. Cells were washed three to four times with PBS between and after incubations with primary and secondary antibodies. 5-Bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium was used as a substrate for alkaline phosphatase to reveal rNIS-specific staining.

**Three-Dimensional Tumor Spheroid In vitro Model.** We used the liquid-overlay technique to grow multicellular tumor spheroids from cultured cancer cells. In our modifications of this technique, spheroids were grown in suspension above a nonadhesive agar (17). Briefly, DU145 cells were placed into tissue culture flasks containing 1% Noble agar and incubated 5 to 7 days at 37°C with 5% CO₂ and 95% humidity. Cell aggregates were transferred to a fresh flask with agar and incubated for an additional 7 days to form spheroids of varying sizes. The spheroids 100 to 150 μm (Fig. 5A) in diameter were isolated by size exclusion and transduced with 10 MOI of Ad-rNIS. Twenty-four hours after transduction, the spheroids were washed and incubated with 20 μCi of 131I during 24 hours. After treatment, all groups were washed and placed in 24-well tissue culture plates containing 0.85% agar and DMEM with 10% FBS. Diameters of all spheroids were measured before and after treatment.

**In vivo DU 145 Xenograft Model of rNIS Gene Therapy.** The animal experiments described were reviewed and approved by the Institution Animal Care Utilization Committee at the Iowa Cancer research Foundation (Des Moines, IA). Forty athymic nude mice were s.c. implanted with 20 × 10⁶ DU145 cells per mouse in their right flank. Mice were divided into four groups: one experimental and three control groups. The experimental group received three intratumoral injections of 10⁸ PFU/mouse of Ad-rNIS at 24-hour intervals between injections. On the fourth day after the first adenoviral injection, 3 mCi of 131I were administered i.p. to each mouse. One control group received three intratumoral injections of 10⁸ PFU/mouse of Ad-rNIS without subsequent injection of 131I. A second control group received three injections of 10⁸ PFU/mouse of adenoviral vector containing herpes simplex virus thymidine kinase followed by 3 mCi of 131I administration. The third control group received 3 mCi of 131I i.p. without any intratumoral injections. After radioactive iodine injection, mice were switched to a diet of soft wet food. Tumor volumes were measured (length × width × height) in mm³. Mice with tumors reaching a volume >1000 mm³ were euthanized.

**RESULTS**

**Production of Adenovirus Containing rNIS Gene.** Recombinant adenovirus containing the rat NIS gene was generated using homologous recombination in 293 cells. After transfection, 24 visible plaques were harvested, grown on 293 cells in 24-well tissue culture plates, and DNA was isolated from viral lysates. The eight positive adenoviral clones were selected using rNIS gene-specific PCR, and viral titer was determined by plaque assay. The recombinant adenoviruses were individually analyzed for rNIS activity in infected DU145 cells. Clone 5 was selected to address our research aim and was designated as Ad-rNIS and further purified twice by plaque assay. Finally, a large-scale production of Ad-rNIS was performed resulting in a purified viral suspension with a titer of 4 × 10¹¹ PFU/mL.

**125I Uptake in Cancer Cells Infected by Ad-rNIS.** To determine the ability of Ad-rNIS to deliver a functionally active rNIS gene to human cells, we used DU145 prostate cancer cells, T47D breast cancer cells, and HCT-15 colon cancer cells. None of these cell lines express endogenously the NIS gene, and no detectable radioiodide uptake occurs in nontransduced cells. In all three cell lines, robust expression of rat symporter gene was observed after adenoviral delivery. Iodine-125 uptake levels in those cell lines were 150 to 200-fold higher in comparison to radioiodide uptake levels in control nontransduced cells. In vivo DU 145 Xenograft Model of rNIS Gene Therapy. The animal experiments described were reviewed and approved by the Institution Animal Care Utilization Committee at the Iowa Cancer research Foundation (Des Moines, IA). Forty athymic nude mice were s.c. implanted with 20 × 10⁶ DU145 cells per mouse in their right flank. Mice were divided into four groups: one experimental and three control groups. The experimental group received three intratumoral injections of 10⁸ PFU/mouse of Ad-rNIS at 24-hour intervals between injections. On the fourth day after the first adenoviral injection, 3 mCi of 131I were administered i.p. to each mouse. One control group received three intratumoral injections of 10⁸ PFU/mouse of Ad-rNIS without subsequent injection of 131I. A second control group received three injections of 10⁸ PFU/mouse of adenoviral vector containing herpes simplex virus thymidine kinase followed by 3 mCi of 131I administration. The third control group received 3 mCi of 131I i.p. without any intratumoral injections. After radioactive iodine injection, mice were switched to a diet of soft wet food. Tumor volumes were measured (length × width × height) in mm³. Mice with tumors reaching a volume >1000 mm³ were euthanized.

**Western Blot Analysis of rNIS Expression in Cancer Cells.** Membrane fractions were isolated from three human cancer cell lines to identify the expression of rat transmembrane NIS protein. Membrane fractions were isolated from nontransduced cells and at 24, 48, and 72 hours after transduction with 50 MOI of Ad-rNIS. No protein bands were revealed in Western blots of nontransduced cells, showing that cross-reaction of rNIS-specific antibody with the other membrane proteins did not occur (Fig. 2). At 24 hours after transduction, multiple bands were detected between Mᵣ 49,700 and Mᵣ 112,000 in DU145, T47D, and HCT-15 cells that represent glycosylated forms of the symporter. NIS is known to be a glycosylated protein and has three Asn-glycosylation sites (25). Additional bands were observed in the high molecular weight area in HCT-15 and DU145 cells, most likely representing dimeric and oligomeric forms of rNIS (15, 25). Nonglycosylated forms of rNIS protein were also observed Mr <80,000 in the cell lines. Variations in posttranslational modifications and partial processing of rNIS

**Fig. 1** 125I uptake in different cancer cell lines after adenoviral delivery of rNIS gene. DU145, T47D, and HCT-15 cells were placed in 24-well plate and transduced with 50 MOI of Ad-rNIS. Forty-eight hours after transduction, cells were analyzed for functional rNIS activity using 125I uptake assay. 1. nontransduced cells; 2. cells transduced with 50 MOI of Ad-rNIS; and 3. cells transduced with 50 MOI of Ad-rNIS and incubated with sodium perchlorate. The data represent the mean based on triplicate experiments (SE was <10%). ∗, P < 0.01.
possibly explain why Western blot analysis revealed different distribution of rNIS-specific bands in the three cancer cell lines tested (29).

**Immunocytochemical Detection of rNIS Protein.** DU145, T47D, and HCT-15 cells were plated into 6-well tissue culture plates, transduced with 50 MOI of Ad-rNIS, and fixed 48 hours after transduction. Heterogeneous distribution of staining was observed in DU145 cells transduced with Ad-rNIS (Fig. 3A). Approximately 70 to 80% of cells were positive for rNIS-specific staining. Some cells were more intensely stained than others, which demonstrated that the level of rNIS expression varied between cells transduced with various MOI of adenoviral vectors. Intracellular localization of rNIS-specific staining was observed mainly in two areas of permeabilized DU145 cells: plasma membrane and the cytoplasm, confirming that rNIS is synthesized in cancer cells and transported to plasma membrane of those cells. Only background staining was observed in DU145 cells infected with Ad-rNIS and incubated with preimmune serum (Fig. 3B). The same pattern of rNIS-specific localization in plasma membrane and the cytoplasm was observed in T47D and HCT-15 cell as shown for DU145 cells (data not shown).

**Effect of 131I on the Growth of DU145 Multicellular Tumor Spheroids Expressing the Rat NIS Gene.** Previously, we determined that 95% of control spheroids survived after 24 hours of incubation with 20 μCi of 131I (17); therefore, a 20-μCi dose was used. The spheroids were divided into four groups: the first group received no treatment, the second group received 20 μCi of 131I for 24 hours, the third group received 10 MOI of Ad-rNIS, and the fourth group received 10 MOI of Ad-rNIS and 20 μCi of 131I for 24 hours. Intact spheroids and spheroids incubated with radioiodine alone increased in size and exhibited normal growth patterns (Fig. 4A, B, D–E). Transduction of spheroids with 10 MOI of Ad-rNIS alone had no nega-

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**Fig. 2** Rat NIS expression in cancer cells transduced with 50 MOI of Ad-rNIS. Membrane fractions were isolated from nontransduced and transduced with 50 MOI of Ad-rNIS DU145, T47D, and HCT-15 cancer cells and analyzed by Western blot analysis. Thirty micrograms of total protein were loaded in each well. Site-directed polyclonal anti-COOH-terminal rNIS antibody was used for rNIS detection. No bands were observed in noninfected cells. Multiple bands were observed in infected cells and represent different forms of rNIS.

**Fig. 3** Immunocytochemical detection of rNIS in DU145 cells. DU145 cells were placed in 6-well plates, transduced with Ad-rNIS, and at 48 hours used for rNIS detection. A. DU145 cells transduced with 50 MOI of Ad-rNIS; magnification, ×100. B. DU145 cells transduced with 50 MOI of Ad-rNIS; preimmune rabbit antibody used as a primary antibody; magnification, ×100.
tive effect on spheroid growth (Fig. 4C). The fourth group was transduced with Ad-rNIS followed by incubation with radioiodine. In this treatment group, 90% (9 of 10) of the spheroids rapidly decreased in size and disappeared within 10 days (Fig. 4F).

**Effective Prostate Cancer Xenograft Treatment after In vivo Transduction with the Ad-rNIS Vector followed by 131I Administration.** To evaluate antitumor effects of the rat NIS in vivo, DU145 human prostate cancer cell xenografts were established in four groups of athymic nude mice (seven to eight mice per group). When tumor volumes reached 200 ± 50 mm³, three consecutive intratumoral injections of 10⁶ PFU of Ad-rNIS every 24 hours were performed followed by i.p. administration of 3 mCi of 131I on day 4. Tumor volume measurement revealed an immediate inhibition of tumor growth after radioiodine injection in the rNIS and 131I treatment group of mice (Fig. 5A). In all control groups, tumors rapidly increased in size with similar growth patterns. Fifty percent of the tumors in control mice reached volumes larger than 1000 mm³ within 13 days, and mice were euthanized according to the protocol. Survival data demonstrated that in the rNIS and 131I treatment group 88% (seven of eight) of mice survived through day 30 (Fig. 5B). In contrast, mice from three control groups demonstrated only 18% survival (4 of 22) at 30 days after radioiodine injection.

**DISCUSSION**

For many years, radioactive iodide therapy has been considered as an effective treatment for well-differentiated thyroid carcinoma (30–32). Successful treatment of thyroid carcinoma is possibly due to 131I accumulation mediated by the NIS expressed in thyroid cells. The 10-year survival rate for middle-aged adults with well-differentiated thyroid carcinoma is 80 to 90% (33). The cloning and characterization of the NIS gene has led to development of a potential new technology for targeted cancer gene therapy. The first step was made by demonstration that nonviral delivery of the rat NIS gene to cells normally not expressing NIS resulted in 125I uptake in transfected cells (14, Fig. 5A). Twenty microcurie of 131I destroy DU145 spheroids expressing rat sodium iodine symporter gene. The spheroids were transduced with 10 MOI of Ad-rNIS. Twenty-four hours after transduction, the spheroids were incubated with 20 μCi of 131I for 24 hours, washed, and placed in 24-well plates containing 0.8% agar with one to two spheroids/well. A and B, control spheroids on day 0. C, control spheroids on day 10. D and F, spheroids incubated with 131I. E and F, spheroids transduced with Ad-rNIS.

![Image of spheroids and tumor volume measurement](image-url)

![Image of tumor growth](image-url)

**Fig. 4** Twenty microcurie of 131I destroy DU145 spheroids expressing rat sodium iodine symporter gene. The spheroids were transduced with 10 MOI of Ad-rNIS. Twenty-four hours after transduction, the spheroids were incubated with 20 μCi of 131I for 24 hours, washed, and placed in 24-well plates containing 0.8% agar with one to two spheroids/well. A and B, control spheroids on day 0. C, control spheroids on day 10. D and F, spheroids incubated with 131I. E and F, spheroids transduced with Ad-rNIS.

**Fig. 5** Antitumor effect of rat NIS expression and radioiodide administration in athymic nude mice. Animals were injected into the right flank with 20 × 10⁶ DU145 cells per mouse. An experimental group received three daily intratumoral injections of 10⁶ PFU of Ad-rNIS followed by i.p. administration of 3 mCi of 131I. On the fourth day, 3 mCi of 131I were administered i.p. to each mouse. One control group received three intratumoral injections of 10⁶ PFU of Ad-rNIS vector without 131I. Another control group received three injections of 10⁶ PFU of Ad-TK vector followed by the administration of 3 mCi of 131I. The final control group received 3 mCi of 131I alone. A, tumor growth (mean ± SE). — — —, Ad-rNIS + 131I; ▲ — ▲, Ad-rNIS; ▼ — ▼, Ad-TK + 131I; — — ▼ , Ad-TK + 131I; — ▼ ▼ ▼ ▼ ▼, B, the survival of mice. — — —, Ad-rNIS + 131I; — ▼ ▼ ▼ ▼ ▼, Ad-TK + 131I; — ▼ ▼ ▼ ▼ ▼, Ad-TK; — • — • — ▼ ▼ ▼ ▼ ▼, 131I; *, P < 0.01.
Our group further demonstrated that cancer cells could be transduced in vitro by a retrovirus carrying the rNIS gene and could be specifically killed by $^{131}$I accumulated in transduced cancer cells expressing NIS. Furthermore, imaging data revealed in vivo accumulation of $^{125}$I in cells expressing NIS in tumor xenografts established in athymic nude mice (12, 34). Spitzweg et al. (13) demonstrated that delivery of the human NIS gene under control of a prostate-specific antigen gene promoter resulted in $^{125}$I accumulation in prostate cancer cells. Unfortunately, both nonviral and retroviral NIS gene delivery resulted in subtherapeutic concentration of radioactive iodine $^{131}$I that did not induce tumor regression (data not shown). Adenoviral vectors provide transient robust expression of transgenes and are very efficient for in vivo gene transfer. Cho et al. (19) demonstrated that functional exogenous human NIS could be detected in a xenografted human glioma after intratumoral injection of recombinant adeno-virus containing the human NIS gene. Spitzweg et al. (21) used prostate cancer (LNCaP cells) xenografts in athymic nude mice to explore the efficacy of NIS gene therapy, using recombinant adeno-virus containing the human NIS gene. Importantly, significant antitumor effect was demonstrated after administration of 3 mCi of $^{131}$I. Their study provided strong evidence that sufficient human NIS activity for a therapeutic radioiodide dose could be achieved within the tumors.

Previously, using a retroviral system, we demonstrated rat NIS gene expression resulted in higher levels of $^{125}$I uptake in cancer cells compared with the human NIS gene (22). In the present study, we therefore used a replication-defective adenoviral vector to deliver the rat NIS gene to cancer cells and to study its antitumor effect. Robust function of the rat symporter was detected in DU145, T47D, and HCT-15 human cancer cell lines infected with Ad-rNIS. All three cancer cell lines successfully transferred functionally active rat symporter to the plasma membrane, resulting in very high levels of $^{125}$I accumulation in those cell lines. Our in vivo data demonstrated immediate inhibition of tumor growth after Ad-rNIS intratumoral injection and $^{131}$I administration. This showed that a therapeutic radioiodide dose was accumulated in tumors expressing the rat NIS gene, whereas this same dose of $^{131}$I in absence of the rat NIS gene had no effect on the growth of tumors in control groups of mice.

Nearly all published reports describing the use of adenoviral vectors for transgene delivery to tumor xenografts established in athymic nude mice have used $10^8$ to $10^{10}$ PFU of adenoviral vectors per mouse (35–41). An important consideration is to achieve a therapeutic effect of treatment with the smallest dose of adenoviral vector possible. In this study, a relatively low dose of Ad-rNIS (total 3 $\times$ 10$^8$ PFU per mouse) was used to achieve a therapeutic effect of treatment. Survival data demonstrated that 100% of experimental mice survived $>$13 days and 88% $>$ 30 days, whereas in control groups, 50% of the animals survived $>$13 days and only 18% survived $>$30 days. No significant toxicity from injected adenovirus or radioiodine was noticed during the observation period. Tumor growth curves for the three control groups were nearly identical (Fig. 5A).

Improving the effectiveness of a treatment will have clinical value only with a concurrent increase in the therapeutic index (42–46). Localized intratumoral accumulation of radioactive iodine should potentially have a greater therapeutic index than external beam radiation therapy. To further increase the therapeutic index, the NIS gene could be combined with limited replication adenoviral vectors or tumor specific expression technology (47–51). Freytag et al. (52–54) recently described a three-pronged approach in which they combined intraprostatic administration of a replication-competent, oncolytic adenovirus containing a cytosine deaminase/herpes simplex virus thymidine kinase fusion gene concomitant with increasing durations of 5-fluorocytosine and valganciclovir prodrug therapy and conventional-dose three-dimensional conformal radiation therapy. In this work, they showed that the therapeutic index was increased by using the combination of suicide genes, restricted adenoviral replication, and radiation therapy.

A potential disadvantage of current adenoviral vectors is the induction of a significant host immune response. T-Cell-mediated response against adenoviral proteins causes a local inflammatory reaction that can result in lysis of the transduced cells and a shorter duration of adenoviral-mediated transgene expression (55). However, in some therapeutic approaches, this problem could be overcome. The NIS gene expression reaches maximal levels at 72 hours. Intratumoral injection of adenovector carrying transgene and $^{131}$I administration at 72 to 96 hours after injection can result in therapeutic effect of treatment before a significant immune response develops. Freytag et al. (54) provided additional support that some gene therapy methodologies are not significantly affected by immunity. In phase I clinical trials for the treatment of locally recurrent prostate cancer, a modified replication-competent adenovirus carrying cytosine deaminase/herpes simplex virus thymidine kinase fusion gene was used. At 2 weeks after injection, expression of foreign genes and tumor destruction at the adenovirus injection site were confirmed by sextant needle biopsy of the prostate. Although viral DNA was detected in blood as far out as day 76, no infectious adenovirus was detected in patient serum or urine (54).

It is not yet known whether the use of the rat NIS gene will be advantageous in comparison with the human gene. Our in vitro data demonstrated higher rat NIS functional activity compared with human NIS functional activity after adenoviral delivery. DU145 cells were transduced with 25 MOI of Ad-hNIS or Ad-rNIS, and 72-hour postinfection $^{125}$I uptake was 4 to 6-fold higher in cells infected with Ad-rNIS. Additional animal studies or clinical trials will be required to verify the value of rat NIS gene for cancer therapy.

We have demonstrated for the first time that the rat NIS gene can effectively induce growth arrest of human tumor xenografts after in vivo adenoviral gene delivery and $^{131}$I administration. The data confirm our hypothesis that the rat NIS gene is an attractive suicide gene candidate for cancer gene therapy. Additional research studying the combined effect of the NIS approach with limited replication competent adenoviral system will be performed.

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