A Probasin Promoter, Conditionally Replicating Adenovirus that Expresses the Sodium Iodide Symporter (NIS) for Radiovirotherapy of Prostate Cancer

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Abstract

The sodium iodide symporter (NIS) directs the uptake and concentration of iodide in thyroid cells. We have extended the use of NIS-mediated radioiodine therapy to other types of cancer, we transferred and expressed the sodium-iodide symporter (NIS) gene into prostate, colon, and breast cancer cells using adenoviral vectors. To improve vector efficiency we have developed a conditionally replicating adenovirus (CRAd) in which the E1a gene is driven by the prostate specific promoter, Probasin and the cassette RSV promoter-human NIScDNA-bGH polyA replaces the E3 region (CRAd Ad5PB_RSV-NIS). *In vitro* infection of the prostate cancer cell line LnCaP resulted in virus replication, cytolysis, and release of infective viral particles. Conversely, the prostate cancer cell line PC-3 (androgen receptor negative) and the pancreatic cancer cell line Panc-1 were refractory to the viral cytopathic effect and did not support viral replication. Radioiodine uptake was readily measurable in LnCaP cells infected with Ad5PB_RSV-NIS 24 hours post-infection, confirming NIS expression. *In vivo*, LnCaP tumor xenografts in nude mice injected intratumorally with Ad5PB_RSV_NIS CRAd expressed NIS actively as evidenced by ^{99}Tc uptake and imaging. Administration of therapeutic ^{131}I after virus injection significantly increased survival probability in mice carrying xenografted LnCaP tumors compared to virotherapy alone. The data indicate that Ad5PB_RSV_NIS replication is stringently restricted to androgen positive prostate cancer cells and results in effective NIS expression and uptake of radioiodine. This construct may allow multimodal therapy, combining cytolytic virotherapy with radioiodine treatment, to be developed as a novel treatment for prostate cancer.

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Keywords

prostate cancer; probasin; adenovirus; sodium iodide symporter; virotherapy; gene therapy

INTRODUCTION

Prostate cancer is the second leading cause of cancer death in men [1]. To date, no uniformly curative therapy for metastatic prostate cancer has been developed. In some malignancies, for which existing treatment regimens are not completely effective, suicide gene therapy and virotherapy strategies targeting the tumor-associated genetic alterations represent rational directions for the development of novel therapeutics [2–4].

The sodium iodide symporter (NIS) is a transmembrane glycoprotein that mediates uptake of iodide into cells, especially thyroid follicular cells [5,6]. The presence of NIS on the basolateral membrane of thyroid cells has been exploited for many years for diagnostic imaging purposes as well as for ablative therapy of differentiated thyroid cancer using radioactive iodide (^{131}I). This non-invasive therapy has proven to be a safe and effective treatment for thyroid cancer, even in advanced, metastatic disease [7,8]. In order to extend the use of NIS-mediated radioiodine therapy to other types of cancer, we have successfully transferred and expressed the sodium-iodide symporter (NIS) gene in prostate, colon, and breast cancer cells, both *in vivo* and *in vitro,* using adenoviral vectors. Our experience with adenovirus-mediated NIS transfer and radioiodine therapy was confirmed in large animal model and has culminated in the opening of a phase I trial for prostate cancer that is currently accruing patients [9–14].

All gene therapy approaches depend upon the ability to deliver therapeutic genes to target cells. However, the limited ability to efficiently transduce tumors with effective levels of therapeutic transgenes has been identified as the fundamental barrier to effective cancer gene therapy [15, 16]. To address this issue, conditionally replicating virus, including adenovirus, have been constructed and their efficacy evaluated [17–19]. Two important strategies towards inducing tumor specificity in replicating viral constructs have been pursued: either viral genes essential for viral replication are expressed and controlled by tumor-specific promoters or, alternatively viral genes are mutated in such a way that expression of specific tumor genes are needed for complementation and viral gene induction. Of these two strategies, transcriptional dependent regulation seems to be the most efficient to date [20].

A second conclusion that can be drawn from recent virotherapy clinical trials is that multimodal therapy, combining virotherapy (i.e. viral mediated tumor cytolysis) with chemo- or radiotherapy may be necessary for more complete tumor eradication as opposed to mono-therapy using virotherapy alone [21]. Novel mechanisms that allow such multimodal approaches towards metastatic prostate cancer are sorely needed and thereby enhance anti-tumor effectiveness are sorely needed.

Our approach towards the current problems associated with virotherapy/gene therapy has been the development of tumor specific, conditionally replicating adenoviral vectors that

also harbor the NIS gene. Transcriptional control of the E1A gene would reduce extratumoral toxicity and by inducing tumor selective replication and tumor lysis. NIS expression will allow for non- invasive imaging and reporting as well as radioiodine mediated therapy. This combination of virus mediated oncolysis and NIS mediated radioiodine therapy has been termed "Radiovirotherapy" [22]. We report here the development of a conditionally replicating adenovirus (CRAd) in which the E1a gene is driven by the prostate specific promoter Probasin, thereby targeting prostate cells, and the transcriptional cassette RSV promoter-hNIScDNA-bGH polyA is inserted at the E3 region, inducing NIS expression in permissive prostate cells. Our results suggest that this CRAd may represent a novel approach to prostate cancer gene therapy.

Materials and Methods

Cell Culture

The Androgen-dependent (LnCaP), Androgen-independent (PC-3) prostate cancer cell line, and the pancreatic cancer cell line Panc-1 were cultured as described [10–12,23]. Adenovirus infection was carried out for four hours in serum free media. Cells were then washed once with PBS and replenished with fresh culture media.

CRAd Construction

The shuttle vector pVQPB is a derivative of the ViraQuest shuttle vector pVQAD5 and was constructed as follows; the E1A gene flanked by a 5′ blunt end and an EcoRI 3′ end was isolated from plasmid pCD512_F2 (a gift from Dr. Richard Vile, Mayo Clinic). The shuttle vector pVQA_PB-NIS [24] was digested with BamHI and blunt ended with T4DNA pol, then digested with EcoRI to release the NIS gene. The resulting vector was then dephosphorylated with CIP and ligated to the E1A gene insert. The human NIS cDNA, under Rous Sarcoma Virus LTR promoter control, was subcloned into an Ad5 genomic vector containing an E3-deleted version (ViraQuest). The pVQPB vector and the NIS containing Ad5 genomic plasmid were recombined to yield the CRAd Ad5PB_RSV-NIS. Cell plaques were grown, purified, and characterized by Vira Quest, North Liberty, IA [24]. The final plaque forming unit (pfu) to viral particle (vp) was 1 to 100.

Cytopathic effect assays

1×10^6 cells were infected at MOI 1. Four days post-infection, cells (triplicates) were photographed under a light microscope at $100 \times$ magnification.

MTS Assay

5×10^5 cells were seeded in 96-well tissue culture plates and incubated at 37°C, 5% CO_2 in triplicate. Twenty four hours after plating, cells were infected at the indicated MOI. At time points, 50μl of CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega Corporation) was applied to each well and incubated at 37°C, 5% CO2 for 2 hours. Wells were then read at the 490nm wavelength using a plate spectrophotometer.

Replication Assay of the CRAd vector

4.4×10^6 cells were plated in 150 cm^2 culture flasks and infected at MOI 1 or MOI 0.1. Seventy two hours post-infection media and cells were collected separately. The virus was then purified from each source using the ViraBind Adenovirus Purification Kit (Cell Biolabs). Viral titers were quantified by plaque assay on HEK 293 cells.

^{125}I Uptake

Uptake of ^{125}I by virus-infected cells was measured as described [25,26]. Briefly, 1×10^6 cells/well were plated on 36-well tissue culture plates and infected at MOI 20. At indicated time points, iodide uptake measurement was performed in triplicate in HBSS supplemented with 10 mM HEPES, pH 7.3, 10 μM NaI, and 1×10^5 cpm of ^{125}I. Control wells were incubated in the same buffer plus 10 μM KClO$_4$. Plates were gently mixed and incubated at 37° C, 5% CO$_2$ for 45 minutes. Following incubation, buffers were aspirated and the cells gently washed once with 4°C 10 mM HEPES, pH 7.3 buffer. Cells were lysed in 1 ml 1N NaOH with shaking for 20 minutes at RT, and lysates were assayed for ^{125}I on a gamma counter.

Animal Experiments

All experimental protocols were approved by the Mayo Foundation Institutional Animal Care and Use Committee.

Subcutaneous Tumor Model

Xenografts derived from the LnCaP and Panc-1 cell lines were established into the right flanks of 4–6-week-old athymic nude Foxn1nu mice (Harlan) by subcutaneous injection of 4×10^6 cells resuspended in 0.125μl media and 0.125μl of BD Matrigel basement membrane matrix (BD Biosciences, Bedford, MA). Mice were maintained on a low iodine diet and T4 supplementation (5mg/l) in their drinking water throughout the duration of the experimentation to maximize radioisotope uptake in the tumor and minimized uptake by the thyroid. The mice were examined daily for tumor development.

Titration of intratumoral virus by QPCR

Mice were xenografted with LNCaP or Panc1 tumors. Once tumor size exceeded 100 mm^3, mice were injected intratumorally with the indicated doses of Ad5PB_RSV-NIS. At day ten post-infection, mice were sacrificed and the tumors harvested. Three tumor samples weighing 30 mg were taken from each tumor and DNA was extracted using the DNEasy tissue kit (Qiagen, Valencia, CA, USA) with overnight lysis of tumor tissue in ATL buffer/ Proteinase K. Each sample was assayed for Ad5PB_RSV-NIS genome copies using the Applied Biosystems TaqMan Universal PCR System according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). Briefly, reactions contained 300 nM of a forward primer GACGGCTACGACTGAATG from nt27837 to nt27854 in the Ad5 genome, 300 nM of a reverse primer complementing RSV sequences 118 bp upstream of the RSV promoter, CCGCTTTTCGCCTAAACACAC, and 250 nM of a TaqMan probe binding to the forward strand of the junction between Ad5 E3 sequences and the RSV sequences, 6′ FAM – ATATCTGGCCCGTACATCGCAGAT – Iowa Black FQ, (Integrated DNA

Technologies, Coralville, IA, USA). Five μl of template DNA was amplified in 25 μl reactions using ABI Prism 7900HT Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Genome copies per milligram of tissue were quantitated using a standard curve generated by amplification of known quantities of viral genomes.

Noninvasive imaging of xenografted tumors

Four mice were subcutaneously engrafted with LnCaP and three with Panc-1 cells as described above. When tumors reached 100 mm^3, mice received one intratumoral (IT) injection of 10^{11} vp of the Ad5PB_RSV-NIS CRAd. *In vivo* micro-SPECT-CT imaging was performed one hour after intraperitoneal injection of 0.5μCi of ^{99}Tc. Animals were anesthetized using a Summit Medical Anesthesia Machine (Summit Medical Equipment Company, Bend, Oregon). Oxygen (2 LPM) was used throughout induction and exam. Induction was performed with 4% Isoflurane using an induction chamber. Mice were kept anesthetized throughout the full measurement by 2% Isoflurane using a nose cone. Images were acquired using a Gamma Medica XSPECT system (Gamma Medica, Inc., Northridge, CA). The SPECT scan was performed with a low energy, high resolution parallel-hole collimator, Field of View of 12.5 cm, with a Reported Resolution of 1 to 2 mm. Sixty four projections, 10 sec/projection, were acquired with a Total Acquisition Time of 13:46 min. The CT Scan was performed with a circular orbit 256. Images were acquired at 80kVp and 0.28 mA with a Slice Thickness of 50μm and a Reported resolution of 43 μm. Images were analyzed using the PMOD Biomedical Image Quantification and Kinetic Modeling Software (PMOD Technologies, Switzerland). The level of ^{99}Tc uptake by the tumor was expressed as tumor activity in mCi.

Efficacy Studies

Mice were subcutaneously engrafted with LnCaP as described above. When tumors were visible, one group of mice was used as control (C), a second group received a single intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp (virotherapy V), and the third group received a single intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp and 4 days later a single intraperitoneal dose of 3mCi ^{131}I (radiovirotherapy V+I). The volume of the tumors was measured twice weekly.

RESULTS

Virus construction

The conditionally replicating adenovirus (CRAd) Ad5PB_RSV-NIS harbors the transcriptional cassette RSV promoter-human NIScDNA-bGH polyA inserted at the E3 region. In this CRAd, the E1a gene is driven by a small composite ARR2-Probasin androgen-dependent prostate-specific promoter (PB) [27].

Conditional replication, oncolysis, and spread of Ad5PB_RSV-NIS

To test the specificity of virus replication, three CAR positive cell lines were selected; the androgen dependent prostate cancer cell line LnCaP, the androgen receptor negative prostate cancer cell line PC-3, and the pancreatic cell line Panc-1.

We assayed the specificity of Ad5PB_RSV-NIS replication using several tests. We first examined the specificity of viral protein expression by Western blot for Probasin-driven E1A and Hexon expression (Not Shown). We next measure the specificity of cytopathic effect (CPE) by light microscopy. Cells were infected in triplicates with Ad5PB_RSV-NIS at MOI 1 and examined four days later for signs of CPE. The morphology of the PC-3 cells was unchanged after infection with Ad5PB_RSV-NIS. However, clear signs of CPE in LnCaP were detected under the same conditions (Figure 1A).

To further test for virus-mediated cell-specific killing, the permissive cell line LnCaP was infected with the Ad5PB_RSV-NIS CRAd at increasing MOIs and then assayed daily by MTS. The results were compared to those obtained with the non-permissive pancreatic cell line Panc-1. The LnCaP cells showed a decreased in viability that was both time and MOI doses dependent (Figure 1B). On the other hand, Panc-1 viability was not affected at all (Figure 1C). Similar negative results were obtained using the androgen-independent prostate cell lime PC-3 (Not Shown). Virus progeny production was measured with the Ad5PB_RSV-NIS CRAd in LnCaP, PC-3, and Panc-1 cells three days post-infection at MOI 1 and 0.1 (Figure 2). The amount of virus produced by the LnCaP cells was 7 orders of magnitude higher than the amount produced by the PC-3 cells and 5 orders of magnitude larger than Panc-1. Moreover, the bulk of viral progeny produced by LnCaP was found in the media indicating that viral infection of LnCaP results in cell lysis with subsequent release of infective viral particles.

Taken together, these data show that replication of the Ad5PB_RSV-NIS virus is stringently restricted to androgen receptor positive prostate cancer cells confirming that the virus is a conditionally replicating adenovirus or CRAd.

NIS expression and radioiodine uptake

The next task was to investigate NIS expression induced by the construct, which is driven by the ubiquitous RSV promoter. CRAd-mediated NIS expression, in prostate and pancreatic cell lines which do not naturally supports NIS expression, was examined by Western Blot (Not shown) and by NIS-mediated radioiodine uptake (Figure 3). Ad5PB_RSV-NIS infected LnCaP cells kinetics of iodine uptake at MOI 20 showed a maximum at 24 hours post-infection followed by a sharp decrease (Figure 3A). Radioiodine uptake was inhibited by $KClO_4$, a well known inhibitor of NIS activity indicting that it was specific for NIS expression [5]. These data indicate that an optimal window in time for NIS expression in permissive cells exists, after which NIS expression declines due to virus replication and induction of CPE. In support of this interpretation, cells infected with the non-replicating virus Ad5PB-RSV, in which the NIS gene under the control of the PB promoter is inserted at the E1A region, maintained radioiodine uptake for three days (Figure 3B). Additionally, Panc-1 cells, which are refractory to Ad5PB_RSV-NIS killing, also supported radioiodine uptake mediated by RSV-driven NIS for three days (Figure 3C) but, as expected, did not express PB-driven NIS (Figure 3D).

Our results demonstrate that Ad5PB_RSV-NIS directs NIS expression in permissive cells before the onset of cytopathic effect but, NIS expression is limited in time by cell death. Conversely, in non-permissive cells NIS is expressed with kinetics and dose response

characteristics consistent with transient expression such as that seen after non-replicating virus infection.

Noninvasive imaging of xenografted tumors

Next, we tested Ad5PB_RSV-NIS ability to direct NIS expression in vivo by imaging. When tumors reached 200 mm^3 a single Ad5PB_RSV-NIS dose of 10^{11} vp of was administered intratumorally. Twenty-four hours later, an intraperitoneal dose of 0.5μCi of ^{99}Tc was administered. NIS-mediated ^{99}Tc uptake was detected with a noninvasive micro SPECT-CT imaging system. Figure 5A shows a classical time lapse uptake in LnCaP and Panc-1 xenografted tumors. One hour after delivery of the radiotracer, uptake can be detected in all animals receiving a ^{99}Tc dose over the stomach due to native expression of NIS in the gastric mucosa, even in the absence of viral injection [5],[28]. The thyroid also concentrates ^{99}Tc representing the major NIS expressing organ. Finally the bladder, is also imaged as a result of clearance of the radioisotope in the urine. However, due to their position in the plane of the image, these organs are not seen in all images. Very minimal ^{99}Tc uptake was detected in the Panc-1 tumor at any time point after viral administration (Figure 4A). On the other hand, figure 4A reveals strong ^{99}Tc uptake by the LnCaP tumor that has been injected with the Ad5PB_RSV-NIS CRAd 24 hours post-infection. Quantitation of the image yielded ^{99}Tc uptake in infected tumors of more than 12% of the injected dose. These data indicate specific NIS-mediated ^{99}Tc uptake in the permissive tumor.

A kinetic curve of ^{99}Tc uptake was constructed using xenografted LnCaP and Panc-1 tumors to determine viral persistence. An intratumoral injection of 10^{11} vp was administered at time 0 to all mice in each group. At indicated time points, 0.5 μCi of ^{99}Tc was administered IP and radiotracer uptake was visualized and quantified by micro SPECT-CT imaging. Strong ^{99}Tc NIS-mediated uptake was sustained for up to one month post-infection in the Ad5PB_RSV-NIS infected LnCaP tumors. After this point, mice were sacrificed due to increasing tumor size. On the other hand, only minimal traces of radioisotope accumulation were observed in the infected Panc-1 tumors (Figure 4B). These data indicate that the Ad5PB_RSV-NIS CRAd directs long term expression of functional NIS in LnCaP tumor *in vivo* and, giving the kinetics of NIS expression, suggest that the Ad5PB_RSV-NIS replicates *in vivo*.

Further prove of *in vivo* CRAd replication was obtained by assaying viral genome copy number by QPCR. LnCaP and Panc-1 xenograft tumors established in nude mice were infected with a single dose of Ad5PB_RSV-NIS at 10^{11} vp. Ten days later, the animals were sacrificed and the tumors were tested for viral genome copy contents. We found that the number of viral genome copies per gram of tumor was 3 log orders higher in the permissive LnCaP tumors than in the non-permissive Panc-1 tumors (Figure 5). Taken together, the data presented in Figures 4 and 5 strongly supports the notion that the Ad5PB_RSV-NIS CRAd replicates intratumorally in permissive tumors.

Radioiodine therapy study in vivo

LnCap xenografts were established in three groups of mice. One group (n=10) of mice was used as control (C), a second group (n=10) received a single intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp (virotherapy V), and the third group (n=10) received a single intratumoral injection of Ad5PB_RSV-NIS at 10^{11} vp and 4 days later a single intraperitoneal dose of 3 mCi ^{131}I (radiovirotherapy V+I). Tumor measurements started the day of virus administration and twice weekly thereafter. Mice were followed for a total of 40 days or until tumor burden was such that animals had to be sacrificed (Figure 6A). In the control group, tumors grew steadily. The high values in the standard errors reflect large variations in tumor growth. Virotherapy treatment slowed tumor growth considerably. As for the control group, high values in the standard errors were observed as a consequence of irregular, albeit greatly decreased, tumor growth. When animal received radiovirotherapy tumor growth was completely arrested. This effect was less variated as attested by the small values of the standard errors. Linear mixed effect models with random intercept and slope were fitted for each group. Pairwise comparisons of the slopes using the likelihood ratio test indicated significant differences among all treatment groups after Bonferroni's adjustment (control *vs* virus p=0.001, control *vs* radiotherapy p<0.001, and virus *vs* radiotherapy p<0.001).

We investigated whether treatment with Ad5PB_RSV-NIS CRAd combined with ^{131}I radiotherapy could increase survival. LnCap xenografts were established in three groups of mice. One group (n=10) of mice was used as control (C), a second group (n=10) received a single intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp (virotherapy V), and the third group (n=10) received an intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp and 4 days later a single intraperitoneal dose of 3 mCi ^{131}I (radiovirotherapy V+I). Mice were followed and the survival times of each group were displayed using the Kaplan-Meier curve (Figure 6B). The end point event was set at tumor burden   1,000 mm^3, while death before end point was considered as censoring. By week 8 all animals in the control group reached tumor sizes 1,000 mm^3. Virotherapy resulted in a net improvement in probability survival over the control group. Thus, while all control animals have died by week 8, the virotherapy group had a probability of survival of 0.5. However, after week 8, survival in this group plunged precipitously. On the other hand, the radiovirotherapy group achieved a 0.5 probability of survival up to 18 weeks, at which point the experiment was ended. The survival times of the three treatment groups were significantly different as indicated by the log-rank test (p=0.001). Assuming proportional hazard, the hazard ratio of virotherapy vs control was 0.360 (95% CI: 0.125---1.040) and the hazard ratio of radiovirotherapy vs control was 0.115 (95% CI: 0.031---0.423). Taken together, these results indicate that the combination of radiotherapy and cytolytic virotherapy was superior to virotherapy (p=0.001) which was a moderate improvement over control.

DISCUSSION

We have reported the use of the probasin promoter to target expression of NIS to prostate cancer cells *in vitro* [29]. Our initial *in vitro* studies with the non-replicating virus As5PB-NIS, in which the PB promoter drives the expression of the NIS, yielded high levels of

membrane-targeted NIS expression in androgen-positive LnCaP prostate cancer cells. We have extended this study here to an *in vivo* mouse model of prostate cancer. Radioiodide imaging of tumor bearing mice revealed targeted expression of NIS following adenoviral infection with substantial ^{99}Tc concentration at the tumor site. Moreover, virotherapy combine with therapeutic ^{131}I treatment significantly increased survival probability in mice carrying xenografted LnCaP tumors compared to virotherapy alone.

Although a number of gene therapy approaches have been developed for many cancers, clinical trials completed to date have fallen short of expectations [30–34] largely due to limited tumor transduction, and this limitation has been defined as the most important fundamental barrier to effective cancer gene therapy [35]. This has been addressed by escalating the viral dose, but this tactic has also been limited in effect when viral vectors, including adenoviral vectors, are used because of increasing toxicity with higher viral doses. Finding ways to surmount these obstacles is critical for the development of clinically useful cancer gene therapy and virotherapy approaches. Our approach to address the problem of low tumor transduction was to permit local vector replication after delivery using a conditionally replicating adenovirus. We have utilized a transcriptional regulation method placing the E1A gene under control of the prostate specific promoter probasin and the NIS gene under control of the powerful, but non-specific RSV promoter. The rationale for this approach was that viral replication, and consequently extended NIS expression, will be limited to permissive cell lines and tumors. In the present study we show that PB restricts viral protein synthesis, cytopathic effect, cell lysis, and infectious viral progeny production to probasin positive prostate cancer cells. All negative control cells used in this study expressed the CAR receptor, yet androgen negative prostate and non-prostate cells did not support oncolysis and/or viral replication. Moreover, the finding that these cell lines supported transient expression of NIS shows that they were indeed infected by the Ad5PB_RSV-NIS thus reinforcing the notion that the PB promoter targeted viral replication solely to probasin positive cells. In vivo, QPCR assay revealed that the number of viral genome was 3 log orders of magnitude larger in the permissive LnCaP tumors than in the non-permissive Panc-1 ten days post-infection. In addition, robust ^{99}Tc uptake, and thus correct NIS expression, was maintained for a month in the established LnCaP tumors while only very limited uptake was observed in the non-permissive Panc-1 pancreatic tumors. Taken together, and since Ad5 does not replicate in mouse tissues, we interpret these results as proof that the Ad5PB_RSV-NIS CRAd is also capable of *in vivo* replication in permissive androgen positive prostate tumors.

The NIS gene must be properly targeted to the cell membrane to be functional [36]. Under the control of the RSV promoter, NIS was correctly targeted to the cell membrane thereby promoting high levels of iodine uptake both in cell tissue culture and xenografted androgen positive prostate tumors. NIS expression provides a major advantage of this construct; its expression can be directly and non-invasively monitored using diagnostic doses of radioiodine or other similar radionuclides.

The limited efficacy observed in clinical trials of cancer gene therapies to date have suggested that combinatorial therapies to treat cancer will likely be more effective and possibly required for complete tumor eradication. The hypothesis developed from this

observation is that attacking tumor cells through different mechanisms of actions may prevent tumor cells from developing resistance to the treatment combination and may more effectively induce immunity against the tumor [21]. Our results show that Ad5PB_RSV-NIS mediated NIS expression improves the therapeutic value of this CRAd by allowing multimodal therapy in a single agent – viral mediated tumor lysis as well as radioiodine mediated therapy. In addition, it has been shown that E1A represses HER-2/neu resulting in induction of apoptosis [37]. In prostate cancer, the androgen receptor interacts with the HER-2/neu signaling pathways and this interaction contributes to the development of metastatic disease [38]. Therefore, PB restricted expression of E1A to prostate tumors may result in HER-2/neu signaling pathway inhibition thus increasing the efficacy of the Ad5PB_RSV-NIS CRAd.

In conclusion, Ad5PB_RSV-NIS CRAd addresses favorably the major hurdles identified in gene therapy clinical trials for anti-cancer treatments. Radiovirotherapy that combines virotherapy with NIS mediated ^{131}I therapy can be further developed based on this novel CRAd which has the potential to impact prostate cancer treatment.

Acknowledgments

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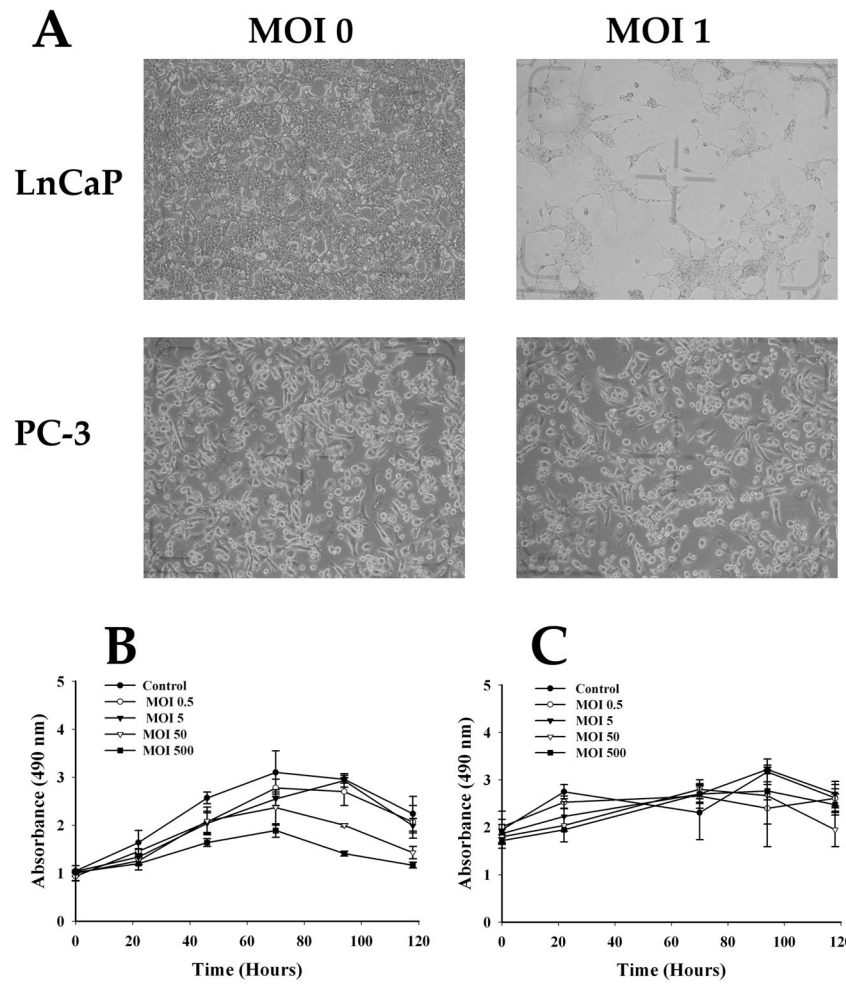
FIGURE 1. Virus Mediated Cell Killing
A) Cytopathic effect. Ad5PB_RSV-NIS-induced cytopathic effect was assessed by light microscopy. B) Virus-mediated cell-specific killing. The prostate cancer cell line LnCaP and the pancreatic cell line Panc-1 were infected with Ad5PB_RSV-NIS at the indicated MOI. At time points cell viability was assayed by MTS.
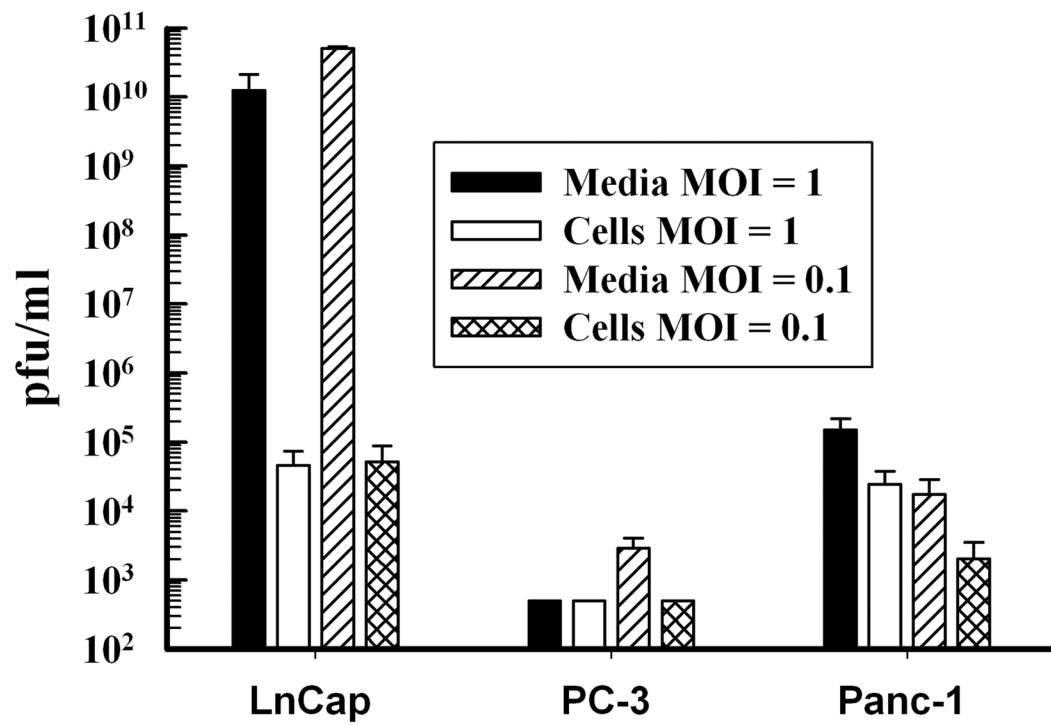
FIGURE 2. Cell Specific Replication of Ad5PB_RSV-NIS
LnCaP, PC-3, and Panc-1 cells were infected with Ad5PB_RSV-NIS at MOI 1 and 0.1.

Viral progeny was isolated from media and cells and titrated by plaque assay.
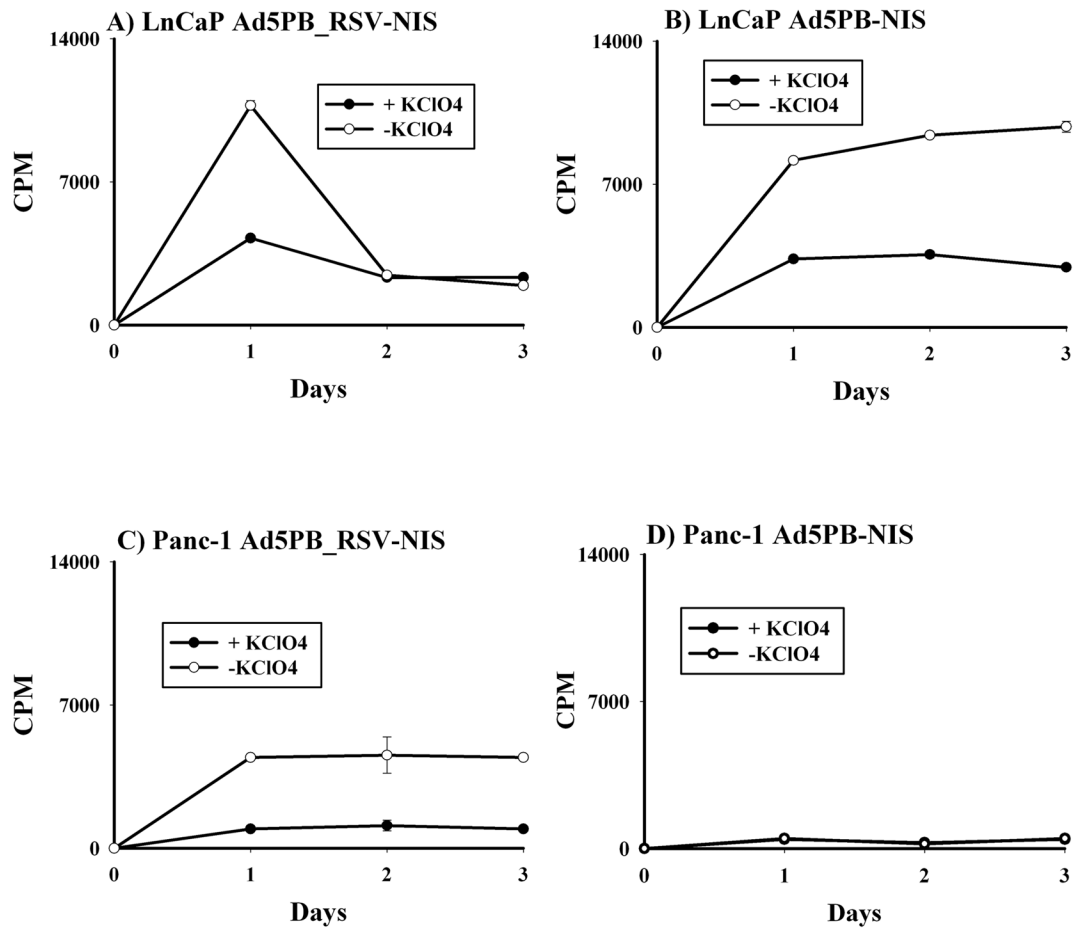
FIGURE 3. Kinetics of NIS-mediated Radioiodine Uptake

LnCaP and Panc-1 cells were infected at MOI 20 with the CRAd or the non replicating virus Ad5PB-NIS in which the E1A region is replaced by the transcription unit PB-NIS-SV40 PolyA. [125]I uptake was measured daily.
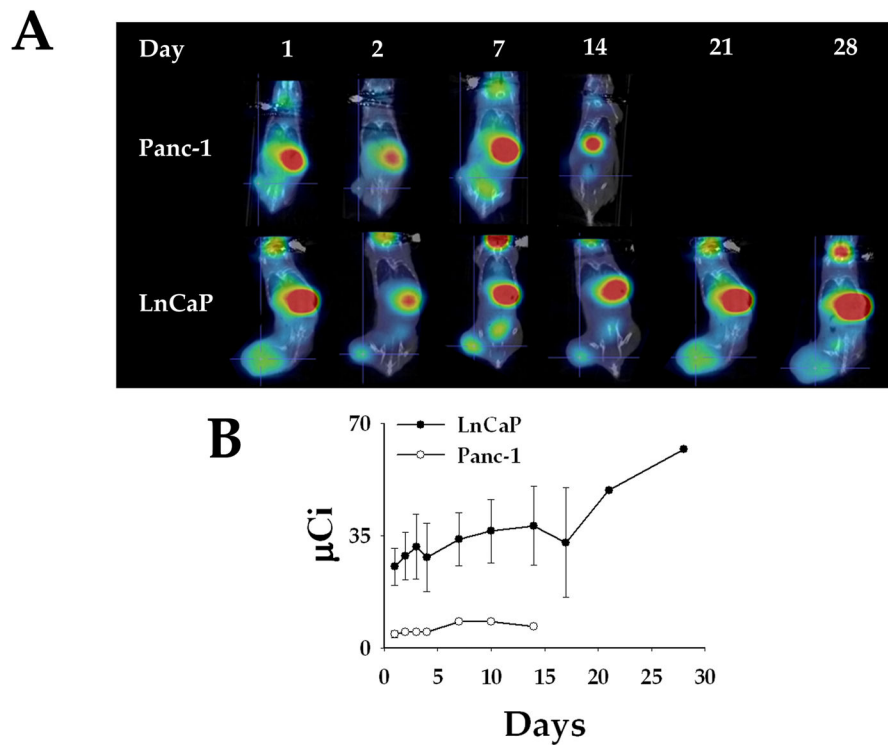
FIGURE 4. Radioiodine Imaging of Xenograft Tumors

A) LnCaP and Panc-1 xenografted tumors were infected with Ad5PB_RSV-NIS at 10^{11} vp. At time points post-infection an injection of 0.5 mCi ^{99}Tc was given IP to all mice. Images were captured using a noninvasive micro SPECT-CT imaging system one hour after radioisotope administration. B) Kinetics of imaging were established by quantifying ^{99}Tc tumor uptake using the PMOD Biomedical Image Quantification and Kinetic Modeling Software (PMOD Technologies, Switzerland). The level of ^{99}Tc uptake by the tumor was expressed as tumor activity in μCi.
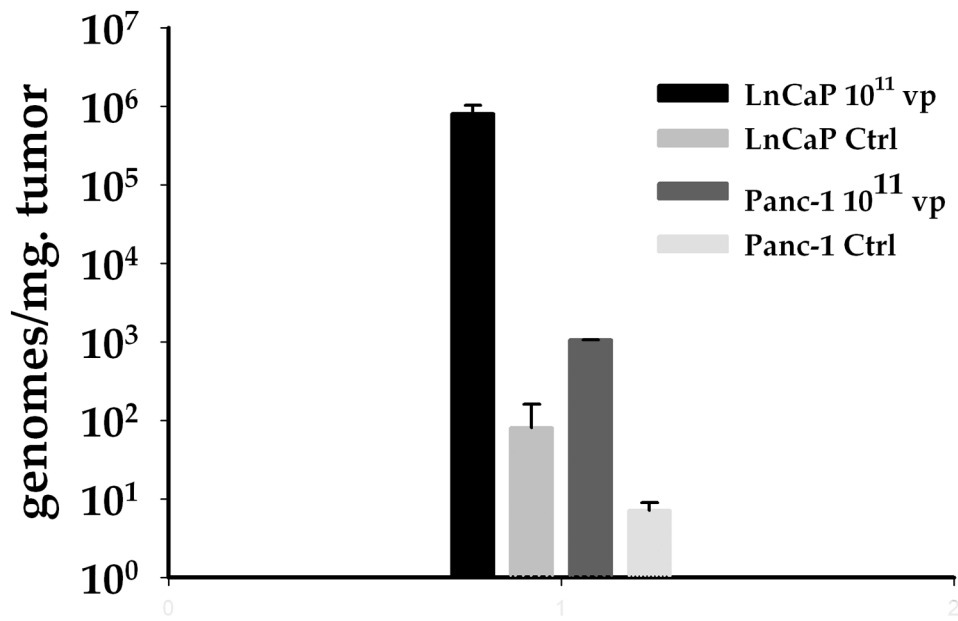
FIGURE 5. Titration of viral genome copies in xenografted tumors
Two LnCaP and two Panc-1 xenografted tumors were infected with Ad5PB_RSV-NIS at 10^{11} vp. Non-infected tumors were used as controls. On day ten the animals were sacrificed and the tumors removed. Viral genome copies were titred by QPCR using three different longitudinal sections (left, center, and right) for each tumor
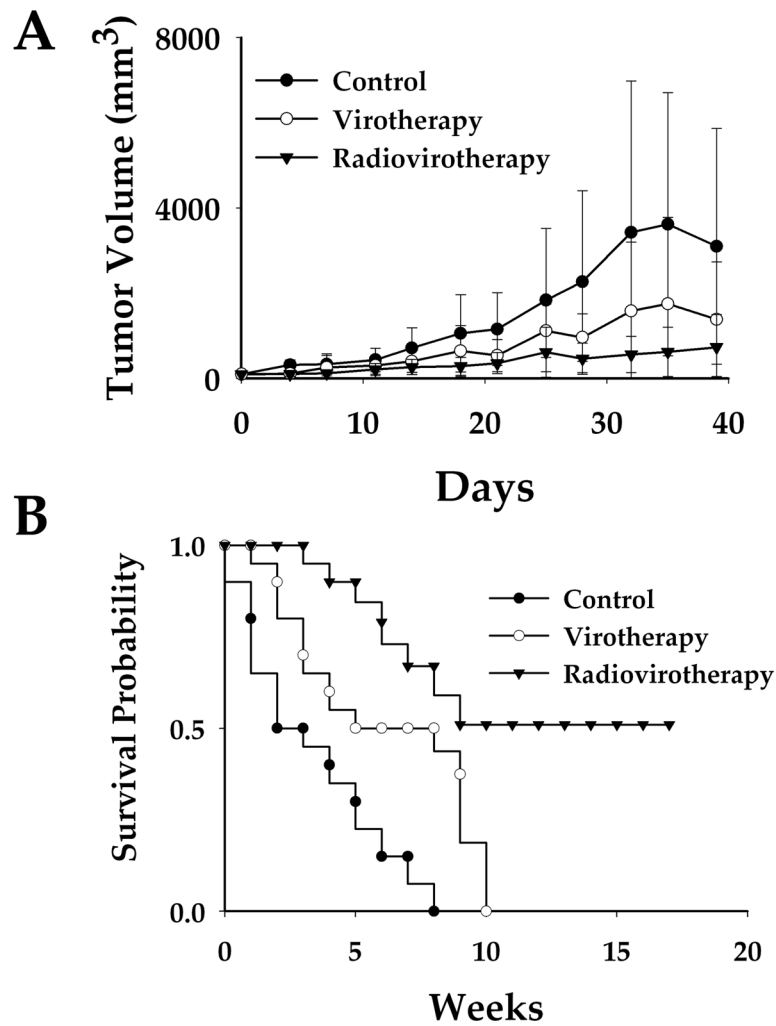
FIGURE 6. Efficacy Studies

Mice were subcutaneously engrafted with LnCaP, one group of mice was used as control (C), a second group received a unique intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp (virotherapy V), and the third group received a unique intratumoral dose of Ad5PB_RSV-NIS at 10^{11} vp and 4 days later a single intraperitoneal dose of 3mCi ^{131}I (radiovirotherapy V+I). For each group n=10. A) Tumor growth kinetics. B) Survival was analyzed according to Kaplan-Meier (t test p=0.001)