Oncolyis of Prostate Cancers Induced by Vesicular Stomatitis Virus in PTEN Knockout Mice

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Abstract

Vesicular stomatitis virus (VSV) is an oncolytic virus which selectively infects and kills cancer cells. The goal of the present study was to determine the safety and efficacy of VSV treatment of prostate tumors that arise in situ in immunocompetent, transgenic prostate-specific PTEN-null (PTEN−/−) mice. Interferon-sensitive VSV(AV3 strain), which expresses luciferase, was injected intraprostatically into tumor-bearing PTEN−/− and control mice and then monitored for tissue bioluminescence over 96 hours. Virus readily dispersed throughout the bodies of mice after only 3 hours; however, it persisted at high levels for >72 hours in PTEN−/− mice, but at relatively low levels and for only ~48 hours in control mice. Plaque assays provided a similar pattern, with much higher concentrations of replicating virus in prostates of PTEN−/− mice than in controls. Transient, low levels of virus were detected in the spleens of both groups. Apoptotic analyses by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining revealed that VSV(AV3) is able to selectively infect and kill prostate cells in PTEN−/− mice, while sparing normal cells in control mice. The primary mechanism for cell kill is apparently apoptotic oncolysis as opposed to neutrophil invasion as has been reported using xenograft models. These results suggest that control of locally advanced human prostate cancer may be achievable through intraprostatic injection and amplification of a safe oncolytic virus, such as VSV(AV3). Cancer Res; 70(4); 1367–76. ©2010 AACR.

Introduction

Prostate cancer is the most prevalent malignancy and second leading cause of cancer-related mortality in North American men, with >186,000 new cases and ~28,000 deaths annually (1). Almost half of all men with clinically localized disease are not cured by surgery or radiotherapy (2, 3). Survival prospects for patients with locally advanced or metastatic prostate cancer are low, and current therapies have side effects that seriously compromise quality of life (2–6). The high incidence of prostate cancer in men >50 years, coupled with the rapidly aging North American male demographic, highlights the need for better management and treatment of this cancer (7).

Treatment with oncolytic viruses, viruses that are able to infect and lyse cancer cells while sparing nonmalignant cells, offers a promising alternative to conventional cancer therapies. In recent years, oncolytic viruses have been tested in various clinical trials for their antitumor targeting capabilities, as they are often able to exploit tumor-specific genetic defects (8–10). Vesicular stomatitis virus (VSV) is an oncolytic virus that can infect and kill cells that have defects in their cellular antiviral immunity, such as the IFN response pathway (11–13). Recent findings have shown that IFN response genes are involved in the pathogenesis of prostate cancer such that downregulation of IFN-inducible genes was observed in ~30% of clinical samples (14, 15).

Previous studies have shown that the selectivity of VSV for malignant cells in comparison with normal cells can be enhanced either by pretreatment with IFNs or by using a mutated form of the virus, referred to as VSV(AV3), which more effectively induces IFN production in infected normal cells (13). Deletion of methionine at position 51 in the matrix (M) protein of VSV renders it more susceptible to the host’s innate immune response. This deletion causes the virus to be safely cleared from normal cells while maintaining its ability to destroy malignant cells, thereby making VSV(AV3) more cancer specific (13, 16–18).

Transplantable human xenografts and transgenic mouse tumor models can be used to test new strategies for the treatment of prostate cancer. Although use of xenografts is limited by the necessity of using immunocompromised animals and need for surgical implantation at orthotopic or subcutaneous sites, transgenic mouse tumor models offer several key advantages for preclinical testing, including the
cancer arises in situ in the target tissue with the appropriate microenvironment, mice possess an intact immune system, and cancers are frequently heterogeneous and, by arising de novo, undergo neoplastic progression similar to that seen in human cancers (19–21). One such transgenic model for prostate cancer is the pAbRR2x PTEN\(^{flox/flox}\) mouse or prostate-specific PTEN-null mice (PTEN\(^{−/−}\)), which leads to de novo formation of prostate tumors. The disease progression of PTEN\(^{−/−}\) mice to prostate cancer is similar to that seen in humans (20, 21). Deletion or mutation of the tumor suppressor PTEN gene has been implicated in many human cancers and has been seen in up to 30% of primary prostate cancers and >64% of prostate metastases, making PTEN an important candidate gene for prostate cancer development and progression (20–23).

The goal of this study was to determine whether VSV(AV3) can differentially infect and kill the prostate tumors that arise in the PTEN\(^{−/−}\) mouse. Our results indicate that this virus is able to selectively infect, replicate, and increase apoptosis in malignant tissue while sparing normal tissue due to a faulty IFN response. Furthermore, cell death is not a by-product of neutrophil infiltration as previously reported in other cancer models (24).

Materials and Methods

**Cell culture.** RWPE-1, LNCaP, and Vero cells were purchased from the American Type Culture Collection. RWPE-1 was maintained in keratinocyte serum-free medium (In Vitrogen) supplemented with bovine pituitary extract and human recombinant epidermal growth factor. LNCaP (Invitrogen) supplemented with bovine pituitary extract 1 was maintained in keratinocyte serum-free medium containing 100 pfu/cell for RWPE-1. MTS (Promega) and 20 μL of VSV(AV3) at 5 × 10\(^8\) pfu/mL were delivered by intraprostatic injection. For quantification of viral uptake and distribution, animals were injected through the i.p. route with 150 mg/kg luciferin (Xenogen) at 3, 6, 24, 48, 72, and 96 h after viral inoculation and imaged with an IVIS100 Imaging System (Xenogen). Data were analyzed using Living Image 2.50 (Xenogen) software.

A small incision was made in the abdomen of PTEN\(^{−/−}\) male mice and 100 μL of VSV(AV3) at 5 × 10\(^8\) pfu/mL were delivered by intraprostatic injection. For quantification of viral uptake and distribution, animals were injected through the i.p. route with 150 mg/kg luciferin (Xenogen) at 3, 6, 24, 48, 72, and 96 h after viral inoculation and imaged with an IVIS100 Imaging System (Xenogen). Data were analyzed using Living Image 2.50 (Xenogen) software.

In **vivo studies.** A small incision was made in the abdomen of PTEN\(^{−/−}\) male mice and 100 μL of VSV(AV3) at 5 × 10\(^8\) pfu/mL were delivered by intraprostatic injection. For quantification of viral uptake and distribution, animals were injected through the i.p. route with 150 mg/kg luciferin (Xenogen) at 3, 6, 24, 48, 72, and 96 h after viral inoculation and imaged with an IVIS100 Imaging System (Xenogen). Data were analyzed using Living Image 2.50 (Xenogen) software.

**Titration of VSV from infected tissue.** Tissues were removed at indicated time points, weighed, and homogenized in 1 mL PBS using a Polytron homogenizer. Serial dilutions were prepared in serum-free medium and added to confluent Vero cells for 60 min. Cells were subsequently overlaid with 1% methyl cellulose (Sigma) and plaques were grown for 48 h. Infected Vero cells were then fixed in 4% formaldehyde and stained with crystal violet. Plaques were counted by visual inspection, and titers were calculated as pfu/mL (28, 29).

**Immunohistochemical staining.** Five-micrometer sections were stained with paraffin-embedded tissues, and tissues were extracted from paraffin as described previously (30). Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assays were performed according to company instructions (Roche). Tissues were stained with primary antibody at the following dilutions: Ki67 (1:100), IFN-regulatory transcription factor (IRF)-3 (1:50), IRF-6 (1:75), IFN-receptor 1 (IFN-R1; 1:1,000), RNaseL (1:1,000), neutrophil regulatory transcription factor (IRF)-3 (1:50), IRF-6 (1:75), IFN-receptor 1 (IFN-R1; 1:1,000), RNaseL (1:1,000), neutrophil regulatory transcription factor (IRF)-3 (1:50), and CD68 (1:100). All antibodies were purchased from Abcam, with the exception of IFN-R1, which was purchased from PBL Biomedical Laboratories. All sections were reviewed by a pathologist.

**Quantitative real-time PCR.** Total RNA from mouse tissue was isolated using the Trizol method (Invitrogen) and reverse transcribed and amplified with IFN-α primers (31) on an Applied Biosystems 7900HT Fast Real-Time PCR System following the SYBR Green PCR Master Mix protocol. Relative quantification of gene expression was performed using rRNA as control.

**Statistical analysis.** GraphPad InStat 3 was used for all statistical analyses. Results are expressed as mean ± SD unless otherwise noted. P < 0.05 was considered statistically significant.
Results

**VSV(AV3) cytotoxicity in prostate cells in vitro.** MTS assay was used to assess the effect of VSV(AV3) infection on the viability of prostate cells propagated in vitro. Two human cell lines representing normal prostate epithelial (RWPE-1) and prostate cancer cells (LNCaP) were tested, as well as a mouse cell line (MPPK-1) derived from prostatic tissue of PTEN<sup>−/−</sup> mice (Supplementary Fig. S1). The results showed that there was an approximate parallel relationship between cell death and increasing viral titer over a 24-hour period for all three cell lines. However, the induction of cell death of VSV(AV3) at a given viral titer was significantly higher in MPPK-1 and LNCaP cells compared with RWPE-1 normal prostate epithelial cells (<i>P</i> < 0.05), suggesting that there was preferential killing of cancer cells by this virus (Fig. 1A). In addition, Western blot analysis of control and MPPK-1 cell lines has shown that treatment with VSV(AV3) led to disappearance of procaspase-3 and pro–poly(ADP-ribose) polymerase proteins in MPPK-1 cells, which further show that these cells undergo apoptotic cell death (Supplementary Fig. S2).

VSV is sensitive to IFN and the mutant AV3 strain is even more IFN sensitive (13, 16, 17). To test this in our system, LNCaP, RWPE-1, and MPPK-1 cells were preincubated for 16 hours with increasing doses of universal type I IFN before being challenged with VSV(AV3). Preincubation of cells with 10,000 IU/mL IFN led to increased cell viability in the RWPE-1 cells. However, in PTEN<sup>−/−</sup>-derived MPPK-1 and LNCaP prostate cancer cells, there was no significant difference in cell death following pretreatment with IFN (Fig. 1B). This showed that the VSV(AV3) strain is highly sensitive to IFN response in normal prostate epithelial cells and only partially in MPPK-1 cells (Supplementary Fig. S3).

**Viral distribution after intraprostatic injections of VSV (AV3) virus into PTEN<sup>−/−</sup> and control mice.** In vivo studies...
were performed to determine VSV(AV3) distribution in both prostate-specific PTEN$^{-/-}$ transgenic mice and control mice. PTEN$^{-/-}$ and normal control mice were injected intraprostatically with 100 μL of $5 \times 10^8$ pfu/mL of VSV(AV3) or UV-inactivated virus (UVI; refs. 24, 32). Viral distribution was monitored by i.p. injection of luciferin followed by bioluminescence measurement over a 3- to 96-hour time period (Fig. 2). Initially after VSV(AV3) infection, viral distribution was seen in the lung and abdomen regions, indicating viral spread in the body. The strongest prostate-localized signal was detected at 3 hours after inoculation with a 6-fold increase in bioluminescence detected in PTEN$^{-/-}$ compared with control mice. In control mice, the prostate-localized signal was decayed ~40% at 24 hours after injection and only trace amounts of bioluminescence were detected at >48 hours (Fig. 2A and C). However, in PTEN$^{-/-}$ mice, a substantial bioluminescence signal was sustained 72 hours after injection, with 18-fold higher bioluminescence intensity measured in comparison with control mice (Fig. 2B and C). These results show that there was a higher initial expression and a greater persistence of VSV(AV3) in the tumor-bearing PTEN$^{-/-}$ relative to control mice.

After administration of the same amount of VSV(AV3) by the i.v. route, the virus was diluted and not clearly detectable. In all cases, VSV(AV3) was sequestered to the spleens of both control and PTEN$^{-/-}$ mice, with only a small amount of bioluminescence detected in ~50% of the prostates of PTEN$^{-/-}$ animals (data not shown). Hence, by injecting virus directly
into the prostates of PTEN−/− mice, there was an apparent amplification of viral load, which led to a distribution of virus throughout the body.

**Presence of live virus in prostatic tissue of PTEN−/− mice.**
To determine whether the observed bioluminescence data correlated with the presence of infectious virus, at 3 to 96 hours after injection, PTEN−/− and control mice were sacrificed and their organs (prostate, spleen, lung, liver, and kidney) were harvested and frozen. Homogenized tissues were titrated by plaque assay (24) to quantify viral delivery and replication within various tissues. Within 3 hours after VSV (AV3) injection in PTEN−/− mice (Fig. 3A), viral titer was highest in prostate (13.0 ± 1.5 log pfu/g) followed by spleen (4.3 ± 0.7 log pfu/g) and lung (2.3 ± 0.3 log pfu/g), with no virus detected in liver and kidney. Live virus in the lung reached its highest point (3.7 ± 0.9 log pfu/g) at 24 hours after intraprostatic injection, whereas in the spleen the viral titer declined and was no longer detectable by 48 hours. By comparison, viral titer in the prostate at 24 hours was slightly reduced (11.7 ± 0.9 log pfu/g) but dramatically increased at 48 hours (30.3 ± 1.5 log pfu/g), suggesting viral replication and amplification in the prostatic tissue of these mice. After 72 hours, there was a marked decline of live virus (1.30 ± 0.33 log pfu/g) in the prostate. In liver and kidney, there was no virus detected at any time, suggesting that these organs were not readily infected or able to support live virus.

In control mice (Fig. 3B), VSV(AV3) was present in both prostate (9.5 ± 0.9 log pfu/g) and spleen (8.7 ± 1.7 log pfu/g) at 3 hours after injection but not in the liver or kidneys. By 24 hours, this initial level of virus was maintained in the prostate (9.1 ± 1.8 log pfu/g) but declined at 48 hours (4.4 ± 1.2 log pfu/g) and at 72 hours (1.9 ± 0.9 log pfu/g) until eventually no virus was detected. This decline in prostate virions is seen earlier in control mice in comparison with PTEN−/− mice.

![Figure 3. Tissue distribution of replication-competent virus. PTEN−/− (A) and control (B) mice were injected with 100 μL of 5 × 10^8 pfu/mL of VSV(AV3). Mice were sacrificed at time points of 3, 24, 48, 72, and 96 h after viral inoculation. Organs (prostate, spleen, lung, liver, and kidney) were frozen and virus titers were determined by plaque assay. Results are presented as log pfu/g ± SEM (n = 3).](cancerres.aacrjournals.org/content/70/4/1367/F3.expansion.png)
mice. These results show that there was enhanced amplification, viral spread, tissue distribution, and persistence of high levels of prostatic VSV(AV3) infection in PTEN<sup>−/−</sup> mice prostates relative to control animals. In addition, there was a higher level of VSV(AV3) detected initially (3 hours) in the spleen of control (8.67 ± 1.67 log pfu/g) mice in comparison with PTEN<sup>−/−</sup> mice (4.3 ± 0.9 log pfu/g), possibly indicating that the control mice sequester the virus for clearance in their spleen. This difference is no longer detectable by 24 hours after infection with 1.67 ± 0.9 log pfu/g in control mice compared with 1.7 ± 0.3 log pfu/g in PTEN<sup>−/−</sup> mice. The plaque assay was validated using real-time quantitative PCR analysis of viral RNA in the organs of both PTEN<sup>−/−</sup> and control mice relative to UVI VSV(AV3)-treated tissues (data not shown).

VSV(AV3) causes preferential killing of prostate cancer cells in vivo while sparing normal prostate tissue. The difference between viral levels and persistence in the prostates of PTEN<sup>−/−</sup> and control mice led us to assess whether the virus was preferentially infecting and killing tumor cells. To test this, VSV(AV3)-infected prostate tissue from both PTEN<sup>−/−</sup> and control mice was collected at various time points, embedded in paraffin, and evaluated for apoptosis by TUNEL assay (Fig. 4A). There was a substantial increase in apoptotic cell bodies observed in the prostates of VSV(AV3)-treated PTEN<sup>−/−</sup> mice compared with virus-treated controls, where virtually no change in apoptosis was observed (Fig. 4B).

To see the effect of VSV(AV3) infection on cell proliferation, prostate tissues were stained with Ki67 (proliferation marker) and positive cells were counted (Fig. 4C and D). Although there was consistently higher proliferation seen in PTEN<sup>−/−</sup> prostates compared with control, there was no significant difference in proliferation detected after VSV(AV3) infection. This increase in apoptosis and lack of difference in proliferation in the tumor-bearing mice correlated with the increased viral titer previously noted (Fig. 3), indicating that active infection of tumor cells with VSV(AV3) is the cause of this increased apoptosis.

Viral-induced cell death in vivo is not associated with neutrophil infiltration in the PTEN<sup>−/−</sup> tumor model. Previous reports on VSV infection using a nontransgenic mouse model indicated that viral delivery was blocked by vasoconstriction and that cell death was associated with neutrophil infiltration (24, 33), implying that tumor cell death was a consequence of a neutrophil-mediated immune response rather than direct viral oncolysis. To test whether this was also the prevalent cell death mechanism in the endogenous, nontransplanted tumors that arise in our PTEN<sup>−/−</sup> model, prostate tissue was collected after VSV(AV3) infection at various time points, stained for the presence of a repertoire of host immune cells, and then evaluated by a pathologist. Figure 5 shows that in both PTEN<sup>−/−</sup> and control mice prostates, there was an initial rise in neutrophil cells as compared with untreated and UVI-treated prostates.
neutrophil penetration at the 6-hour time point was not significantly different ($P > 0.05$) between tumor-bearing (23 ± 6 cells per time point) and control mice (30 ± 7 cells per time point), and this peak quickly diminished by 24 hours in both PTEN−/− mice (10 ± 6 cells per time point) and controls (8 ± 4 cells per time point). These results suggest that the differences seen in apoptosis (Fig. 4) are not correlated with neutrophil infiltration (Fig. 5A).

When prostate tissues of PTEN−/− and control mice were stained for the presence of macrophages, which, like neutrophils, are phagocytic, there was a marked increase in their number at 24 hours (79 ± 21 cells per time point) and at 72 hours (72 ± 28 cells per time point) in PTEN−/− mice, but almost undetectable and unchanged number of macrophages was seen in the control prostate tissue (Fig. 5B). This macrophage increase may reflect a response to the increasing number of apoptotic tumor cells for which macrophages are recruited to carry out phagocytosis.

**Effects of VSV(AV3) on IFN pathway in vivo.** Because VSV infection leads to activation of the IFN response pathway expression, prostates of control and PTEN−/− mice challenged with VSV(AV3) infection were stained for various components of the IFN pathway such as IFN-R1α, RNaseL, IRF-3, and IRF-7 (Fig. 6A). Immunohistochemical analysis of the prostates of control mice after VSV(AV3) injection shows an increase in staining of all of the mentioned IFN pathway components, showing that there is an intact initial IFN response toward the virus in the prostate tissues of control mice. However, in PTEN−/− prostates, there was no change observed over time in any of the IFN pathway components as scored by a pathologist. This correlates with the increase propagation of virus as seen over time in prostate tumors of PTEN−/− mice (Fig. 3A).

Additionally, mRNA levels of IFN-α present in prostate tissues of VSV(AV3)-infected PTEN−/− and control mice were checked and compared by quantitative PCR analysis. Figure 6B shows that in control prostates treated with VSV(AV3), there is an ~500-fold increase in IFN-α transcription by 48 hours. However, in PTEN−/− prostates treated with the virus at the same time point, there is only an ~40-fold increase in IFN-α mRNA levels. These data suggest that there is a partial IFN response to viral infection in PTEN−/− mice that permits further viral infection and oncolysis of tumor cells (Fig. 3A).

**Discussion**

The goal of any viral therapy for treatment of cancer is to efficiently kill primary and metastatic cancer cells while sparing normal cells (34, 35). Prostate cancer is a good candidate
for viral therapy because there are various technically easy routes (e.g., transurethral, transperineal, and transrectal) for viral delivery (36). Here, the effect of oncolytic virus VSV(AV3), which replicates in IFN-defective cells, has been studied in the prostate-specific PTEN−/− mice model (20). Studies show that ∼75% of tumor cells and 30% of prostate cancer tumors have a defective IFN response, making VSV a useful tumor-selective therapy (11, 12, 15). Additionally, because the primary hosts of VSV are rodents, cattle, horses, and swine, patients living in nonendemic areas do not have neutralizing antibody titers to the virus. VSV rarely infects humans and, in rare cases of infection, presents mild flu-like symptoms, making VSV(AV3) a relatively safe therapeutic agent (37, 38).

To show that VSV(AV3) was able to infect human and mouse prostate cell lines, three cell lines representing normal prostate epithelial cells (RWPE-1), human prostate cancer cells (LNCaP), and mouse prostate cancer cells (MPPK-1) were tested. As shown in Fig. 1, there was a direct correlation between increased viral titer and decreased cell survival in PTEN−/−-derived cell lines (Supplementary Fig. S1) compared with nonneoplastic control cells. These results are consistent with the findings of others (25, 39), although the effects of VSV(AV3) on RWPE-1 and MPPK-1 cells have not been previously shown.

An underlying assumption for using VSV(AV3) for viral therapy is that tumor cells have a defective antiviral host response, specifically in activation of the IFN pathway. To test this, both prostate cancer cell lines (LNCaP and MPPK-1) and noncancerous prostatic cells (RWPE-1) were pretreated with IFN. Our results (Fig. 1B) indicated that due to a defective IFN response, there was no change in the death of prostate cancer cells (LNCaP and MPPK-1), whereas pretreatment of RWPE-1 normal prostate epithelial cells with IFN led to an increase in cell survival. These results confirm previous reports that the mutated M protein in VSV can restrict its infectivity to cancer cells with a malfunctioning IFN response (13, 39).

It was evident from bioluminescence data that, after in vivo administration of VSV(AV3) via direct injection into the prostate, the virus is quickly distributed throughout the body (Fig. 2). This phenomenon is seen in both control and PTEN−/− mice and, as expected, there is a higher viral infection in the prostate by 24 hours, the original site of viral injection. Based on both luminescence and plaque assay data (Figs. 2 and 3), the level of VSV(AV3) infection
and replication in PTEN−/− mice is higher than that of non-tumor-bearing control mice, with an amplification of viral load detected between 24 and 48 hours in PTEN−/− mice.

TUNEL staining of paraffin-embedded prostate tissue from control and PTEN−/− mice after infection indicated high levels of apoptosis in tumor-bearing mice, with very low levels of apoptosis observed in control mice (Fig. 4). The presence of apoptotic cells in control prostate at earlier time points may be due to a low level of viral killing (39) or the trauma of the injection process. The apoptotic cell count peaked at 48 hours after infection in the prostates of PTEN−/− mice. Interestingly, by 96 hours after infection, there was a decrease in apoptotic cells, together with morphologic changes in the prostate tissue, suggesting cell loss through apoptosis (Fig. 4).

It has previously been reported that intratumor injection of VSV(AV3) in BALB/c mice with s.c. tumors leads to tumor cell death through an indirect mechanism, which was linked to neutrophil infiltration (24). Neutrophils originate from WBCs, are phagocytic, and typically act as a first response to pathogens (40). To test this in our model, prostate tissues from both control and PTEN−/− mice, at various times following VSV(AV3) injection, were stained for neutrophils. Our results (Fig. 5A) show that although there was some initial neutrophil infiltration in prostates of control and tumor-bearing mice, there is no evidence that this increase in neutrophil presence correlated with an increase in cell death observed in PTEN−/− prostates. This indicates that neutrophil infiltration did not play a major role in VSV-mediated tumor cell death in the PTEN−/− model. However, with respect to other immune system parameters such as macrophages, there was a significant difference detected after VSV(AV3) injection (Fig. 5B). Previous studies have shown that macrophages play an inhibitory role in viral replication by direct phagocytic clearance of viruses (41, 42). In the PTEN−/− model, an increase in macrophage infiltration occurred after a surge of viral replication, which takes place at 3 hours and then again at 48 hours after viral administration (Fig. 3A), coincident with maximum apoptosis. Because one of the main functions of macrophages is to remove dead cells, this likely explains the increase in macrophage count between 48 and 72 hours after viral infection in these prostate tumors.

VSV infection of normal cells leads to production of IFN-α/β, a primary host innate defense system. IRF-3 and IRF-7 play key roles in transcription of IFN-α/β, which, on production, signals neighboring cells through IFN-Rα/β (43). Binding of IFN-α/β to its receptor activates a new chain of reactions where eventually RNaseL is activated. Activated RNaseL degrades all RNA, thus causing inhibition of viral replication. Therefore, an increase in RNaseL, IRF-3, IRF-7, and IFN-Rα on VSV infection was anticipated and confirmed in the case of control mice injected with VSV(AV3). This was further confirmed through quantitative PCR analysis, showing an increase in IFN-α transcription (Fig. 6). Conversely, in tumor-bearing mice, there was no change detected in the IFN-Rα, IRF-3, IRF-7, and RNaseL protein or IFN-α transcription levels on viral infection. Thus, due to a disrupted IFN response in the PTEN−/− prostates, VSV(AV3) is able to infect cells and increase cell death (Fig. 4).

In conclusion, we have found that in an immunocompetent host, intraprostatic tumor VSV(AV3) injection enables viral replication and amplification sufficient to selectively kill cancer cells while sparing normal cells. The primary mechanism for cell kill is apparently apoptotic oncolysis due to a defective IFN response, as opposed to neutrophil invasion as has been reported using xenograft models (24). These results suggest that control of locally advanced prostate cancer in humans (44) may be achievable through injection of a safe oncolytic virus, such as VSV(AV3), directly into a patient’s prostate tumor.

Disclosure of Potential Conflicts of Interest

No conflict of interest exists, except for J. Bell, cofounder of Jennerex Biotherapeutics, a company involved in development of oncolytic virus therapeutics.

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