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Combined VSV Oncolytic Virus and Chemotherapy for Squamous Cell Carcinoma

Chih-Kwang Sung, MD, MS; Bryan Choi, MS; Georges Wanna, MD; Eric M. Genden, MD; Savio L. C. Woo, PhD; Edward J. Shin, MD

Objectives: Vesicular stomatitis virus (VSV) is a negative-strand ribonucleic acid (RNA) virus that replicates specifically in tumor cells and has oncolytic effects in a variety of malignant tumors. We previously demonstrated recombinant VSV vectors incorporating viral fusion protein (rVSV-F) and interleukin 12 (rVSV-IL12) to have significant antitumor effects against squamous cell carcinoma (SCC) in a murine model. Here we evaluate the potential to combine a potent chemotherapeutic agent for SCC (cisplatin) with rVSV-F and rVSV-IL12 to improve efficacy.

Study Design: In vitro, three SCC cell lines were tested using rVSV-F and rVSV-IL12 with cisplatin, monitoring viral replication and cell survival. In an orthotopic floor of mouth murine SCC model, intratumoral injections of virus combined with systemic cisplatin were tested for tumor control and animal survival.

Results: In vitro, virus and cisplatin combination demonstrated rapid replication and enhanced tumor cell kill. Human keratinocytes were unaffected by virus and cisplatin. In vivo, combined rVSV-F with cisplatin reduced tumor burden and improved survival ($P = .2$ for both), while rVSV-IL12 monotherapy had better tumor control ($P = .06$) and survival ($P = .024$) than combination therapy.

Conclusions: Addition of cisplatin did not affect the ability of either virus to replicate in or kill murine SCC cells in vitro. In vivo, combination therapy enhanced rVSV-F antitumor activity, but diminished rVSV-IL12 antitumor activity. Combination therapy may provide useful treatment for SCC with the development of more efficient viral vectors in combination with different chemotherapy agents or immunostimulatory agents.

Key Words: Oncolytic virus; vesicular stomatitis virus; VSV; rVSV-F; rVSV-IL12; chemotherapy; cisplatin; squamous cell carcinoma; murine.

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INTRODUCTION

In 2006, head and neck cancer had an estimated incidence of 30,990 new cases and 7,430 associated mortalities in the United States, with the vast majority due to squamous cell carcinoma (SCC).[1] Even with advances in treatment, tumors still recur in up to 50% of patients.[2] In unresectable recurrent disease, chemotherapy is the standard treatment option, but is usually limited to palliative intent. Median survival for these patients is 6 to 9 months. In patients with tumors refractory to chemotherapy, median life expectancy drops to 3 months.[3] In light of the poor outlook for patients with recurrent disease, additional effective local-regional therapies are clearly required for the treatment of SCC.

Conditionally replicating viruses targeted to tumors are being developed as a novel class of oncolytic agents. Vesicular stomatitis virus (VSV) is a negative-strand ribonucleic acid (RNA) virus with inherent specificity for replication in tumor cells due to the attenuated antiviral response in these cells. The principal antitumor activity of oncolytic viruses derives from their replication within tumor cells, which results in cell apoptosis and production of progeny virions that can spread to adjacent tumor cells. VSV is a potent oncolytic virus as demonstrated in several preclinical tumor models, including glioma, hepatocellular carcinoma, breast carcinoma, and melanoma. We previously demonstrated recombinant VSV vectors incorporating genes coding for viral fusion protein (rVSV-F) and interleukin 12 (rVSV-IL12) to have significant antitumor effects against squamous cell carcinoma (SCC) in an or-

From the Department of Otolaryngology–Head and Neck Surgery (C.-K.S., B.C., G.W., E.M.G., E.J.S.), and the Carl C. Icahn Institute for Gene Therapy and Molecular Medicine (S.L.C.W.), Mount Sinai School of Medicine, New York, New York, USA.

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Send correspondence to Edward J. Shin, MD, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1189, New York, NY 10029, U.S.A. E-mail: entshin@yahoo.com

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thotopic murine model (Shin et al.).[4,5] We found that rVSV-F replicated efficiently in cultured human and murine SCC cells, whereas normal human and mouse keratinocytes were refractory to the vector. When multiple doses of the vector were injected intratumorally into an orthotopically implanted single SCC nodule in mice, rVSV-F and rVSV-IL12 significantly reduced tumor burden without apparent host toxicity, which led to prolongation of animal survival. Other oncolytic viruses have been studied in combination with chemotherapy to enhance cytotoxic effects. The use of combined therapies that act through different mechanisms may discourage the emergence of treatment-resistant disease. Cisplatin has been the treatment of choice for patients with recurrent or metastatic SCC for two decades.[6] Combination therapy with the addition of systemic cisplatin or 5-fluorouracil following direct intratumoral injection of the oncolytic adenovirus ONYX-015 resulted in clinical regression of head and neck SCC tumors in a phase II trial.[3]

The goal of this study was to investigate the in vitro and in vivo efficacy of recombinant VSV vectors in combination with chemotherapy in the treatment of head and neck SCC. In particular, we examined whether such combination therapy enhances tumor control and survival when compared to single modality treatment with viral vector or cisplatin alone.

MATERIALS AND METHODS

Cell Lines

BHK-21 cell line (used in virus generation) was obtained from American Type Culture Collection (Manassas, VA) and maintained in 10% fetal bovine serum (FBS)-Dalbecco's Modified Eagle's Medium (DMEM) (Mediatech, Herndon, VA). Two human squamous cell carcinoma cell lines, SCC 09 and SCC 38, were both kind gifts from Dr. Dianne Duffey. Murine squamous cell carcinoma cell line SCC VII was a kind gift from Dr. Richard Wong and Dr. Bert W. O'Malley, Jr. The SCC cell lines were grown in DMEM containing 4.5 g/liter glucose + 2 mmol/L L-glutamine, 0.01 non-essential amino acids, 10% FBS and 100 units/mL penicillin and 100 gm/mL streptomycin. Human keratinocytes (HuK) were obtained from Gibco (Invitrogen Corporation, Carlsbad CA). These cells were maintained in defined keratinocyte-SFM (serum-free keratinocyte medium) (Gibco).

Generation of Viruses

The recombinant rVSV-F virus was generated by the methods described in Ebert et al. (2004).[7] Briefly, the plasmid was constructed by PCR amplification of NDV/F, followed by PCR site-directed mutagenesis to form mutated plasmid NDV/F(L289A). The mutated plasmid was then cloned and incorporated in the VSV by reverse genetics to form rVSV-NDV/F(L289A) (rVSV-F); rVSV-IL12 virus was generated by methods previously described by Ebert et al. (2003).[8] IL12 exists as a heterodimer composed of the p35 and p40 subunits. A plasmid containing the fragment murine p40-internal ribosome entrysite-p35 was previously developed and used in other adenoviral constructs.[8] This fragment was released and cloned 3′ of the G protein gene in pVSV. Sequencing confirmed the correct nucleotide sequence. Recombinant VSV-IL12 viruses were generated using the reverse genetic technique.[9]

Assessment of In Vitro Viral Infection With rVSV-F or rVSV-IL12 in Combination With Cisplatin

Murine squamous cell carcinoma cell line SCC VII and human keratinocyte line HuK were used for all of the in vitro studies. In addition, human squamous cell carcinoma lines SCC 09 and SCC 38 were used for the in vitro cytotoxicity studies.

For the quantitative evaluation of viral replication, 1×10^6 cells were plated, left overnight, and then infected (day 0) with rVSV-F or rVSV-IL12 at a multiplicity of infection (MOI) of 0.01 (one virus to 100 cells) with and without the addition of cisplatin to a concentration of 0.1 μg/mL. Supernatants were harvested at multiple time points (0, 4, 24, and 48 hours postinfection). Each time point was tested in triplicate. RNA from the culture supernatants was harvested using the QIAamp viral RNA purification kit (Qiagen, Valencia, CA) and analyzed for the concentration of genomic VSV RNA by real-time reverse transcriptase-polymerase chain reaction assays (RT-PCR) using the Roche LightCycler (Roche, Indianapolis, IN) with specific primers. Experiments were performed on two separate occasions.

For viral and chemo-cytotoxicity, 5×10^4 cells were plated, left overnight, and then infected (day 0) with rVSV-F or rVSV-IL12 at a MOI of 0.01 with or without the addition of cisplatin (1.0 μg/mL in SCC VII and HuK, 0.3 μg/mL for SCC 09 and SCC 38). The cytotoxic effects on the cells of each trial were quantified by a 3-[4,5-dimethythiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay (Roche) and expressed as a fraction of mock-infected cells at each time point. Each point was tested in triplicate, and the experiments were repeated to ensure consistency of results. The efficacy of each treatment condition was compared using the Student t test.

In Vivo Viral Quantification via Plaque Assay

For the in vivo studies, the floor of mouth tumor model was conducted in murine species C3H/HeJ. Six-week-old female C3H/HeJ mice were used in accordance with guidelines for animal use and care established by the Institutional Animal Care and Use Committee. This orthotopic model was chosen because it has been characterized to be aggressive and reflective of the human counterpart.[10] On day 0, syngeneic SCC VII cells (5×10^5) were transcutaneously implanted into the floor of mouth at the level of the mylohyoid. Five days post tumor implantation, rVSV-F and rVSV-IL12 were injected intratumorally at doses of 1×10^7 and 5×10^8 pfu/kg, respectively, with PBS as a control. At 30 minutes and 1, 2, 3, 5, and 7 days, mice were sacrificed, and tumors were harvested and snap frozen at –80°C. BHK-21 cells were seated in six well plates at a density of 3×10^5 cells per well. The following day, the tumors were homogenized in PBS with $CaCl_2$ (1 mmol/L) + $MgCl_2$ (1 mmol/L). The resulting supernatant was diluted from 1:10 to 1:1,000,000 in increments of 10. Each dilution was applied to the BHK-21 cells and incubated at 37 ± 2°C with 5 ± 2% CO_2 for 30 minutes. The supernatant was then removed and each well was treated with Eagle's Minimum Essential Medium (EMEM) supplemented with 2% L-glutamine and 10% FBS contaning 1% agarose. The following day, the agarose was removed and the cells were stained with crystal violet. Plaques were counted and resulting viral titer calculated. Tumor implantation and plaque assay were done in triplicate and duplicate, respectively.[8]

Assessment of In Vivo Intratumoral Infection With rVSV-F or rVSV-IL12 in Combination With Cisplatin in Murine Squamous Cell Carcinoma VII Floor of Mouth Tumors

C3H/HeJ mice were implanted with SCC VII cells as described above. Tumor area was determined by the greatest tumor height and width. By day 5, tumors grew to an average diameter

of 5 to 6 mm in all animals. Daily direct intratumoral injections were then administered for 5 consecutive days (days 5–9). Each tumor was injected with rVSV-F or rVSV-IL12 at 1×10^7 plaque-forming units in 15 μL of phosphate-buffered saline (PBS). Beginning on day 6, daily intraperitoneal injections of cisplatin at 1 mg/kg were administered for 5 consecutive days (days 6–10). Control animals were injected with PBS, cisplatin alone, or virus vector alone. The end points examined were tumor size (using Student t test to compare study groups) and animal survival (Kaplan-Meier survival curves were compared using log-rank test using the Graph Pad Prism 3.0 Program, GraphPad Software, San Diego, CA).

RESULTS

In Vitro Replication

In this study, rVSV-IL12 and rVSV-F with concomitant administration of cisplatin replicated preferentially in murine squamous cell carcinoma cells compared to human keratinocytes (Fig. 1). In SCC VII cells, replication approaches a plateau of 1×10^8 viral ribonucleic acid (RNA) copies within 48 hours, which is 1,000 times greater than that found in human keratinocytes (At 48 hours $P = .036$ for rVSV-IL12, $P = .028$ for rVSV-F). rVSV-IL12 replication is not significantly affected by the presence of cisplatin ($P = .126$ at 48 hours).

In Vitro Cytotoxicity

Recombinant VSV-IL12 and rVSV-F administered in combination with cisplatin both preferentially killed murine squamous cell carcinoma cells when compared to normal human keratinocytes ($P < .001$ for both viruses) (Fig. 2). The human keratinocyte population remained at an essentially steady level. Addition of cisplatin to virus increased cytotoxicity in SCC VII compared to virus alone ($P < .001$ for both rVSV- IL12 and rVSV-F). Addition of cisplatin to rVSV-F did not significantly affect cytotoxicity in the human squamous cell carcinoma SCC 38 at 72 hours; however, cytotoxicity was slightly decreased in SCC 09 ($P = .358$ for SCC 38, $P = .016$ for SCC 09).

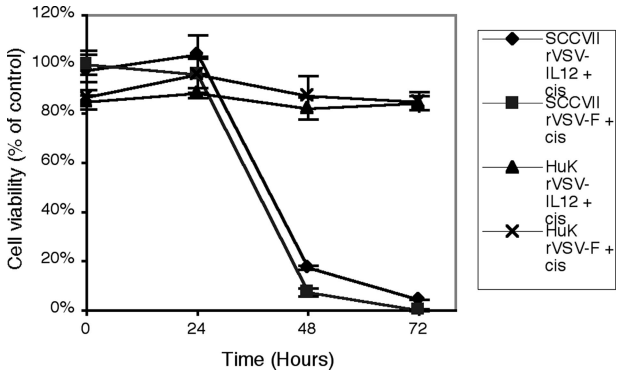

Fig. 2. Cell viability expressed as percentage of control. Cell viability decreases significantly over time in the squamous cell carcinoma (SCC) VII tumor cells, but not in the human keratinocytes (HuK) when treated with virus in with cisplatin (At 72 hours, $P < .001$ for both rVSV-IL12 and rVSV-F). Cell viability in SCC VII cells decreases significantly with the addition of cisplatin to virus versus virus alone (At 72 hours, $P < .001$ for both viruses). Error bars represent SEM.

Notably, cisplatin did not increase the toxicity of the rVSV-F to human keratinocytes (Fig. 3).

In Vivo Viral Replication

Viral titers in tumors were quantitatively measured via plaque assays at 30 minutes, 1, 2, 3, 5, and 7 days after intratumoral injection (Fig. 4). There was an increase of viral yield by nearly 2-log at 2 days versus 1 day for rVSV-F injection, indicating successful intratumoral viral replication. For rVSV-IL12, initially high viral titers diminished by two to three orders of magnitude each day from day 1 to 3, and to undetectable levels after 5 days, suggesting suppression of viral replication by host immune response.

In Vivo Tumor Size

Treatments with rVSV-F or rVSV-IL12 combined with cisplatin (n = 8 and 7, respectively) reduced tumor

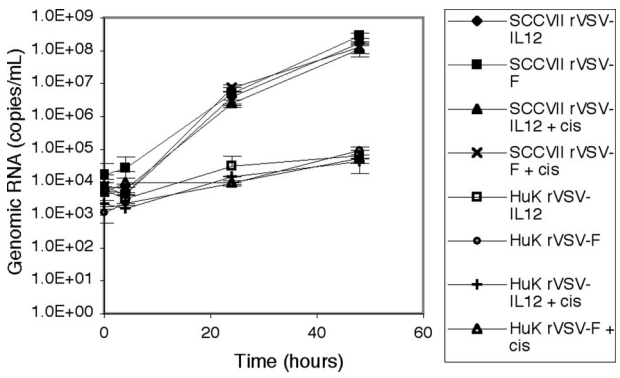

Fig. 1. Viral replication expressed as amount of ribonucleic acid (RNA) copies/mL on a $\log_{10}$ scale. In the SCC VII cell line, viral replication approaches a plateau of 1×10^8 within 48 hours after infection with rVSV-IL12 and rVSV-F with or without cisplatin, and is 1,000 times the replication in human keratinocytes. At 48 hours, rVSV-IL12 with cisplatin and rVSV-F with cisplatin replicate to higher levels in SCC VII cells than in human keratinocytes ($P = .036$ and 0.028, respectively). Addition of cisplatin did not affect viral replication in SCC VII. Error bars represent SEM.

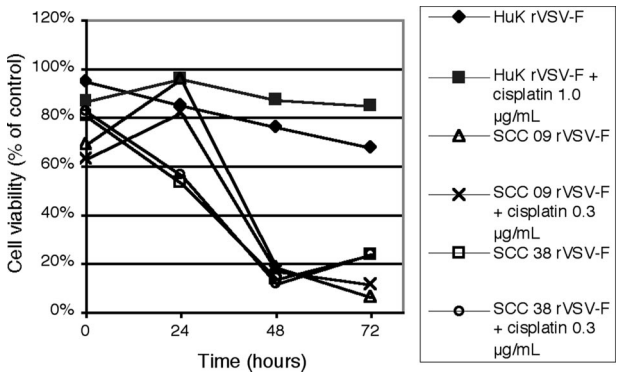

Fig. 3. Cell viability expressed as percentage of control. Cell viability decreases over time in human squamous cell carcinomas cell lines squamous cell carcinoma (SCC) 09 and SCC 38 when treated with rVSV-F with or without cisplatin 0.3 μg/mL. The curves for human keratinocytes (HuK) treated with rVSV-F with or without cisplatin 1 μg/mL are included for reference. Addition of cisplatin did not significantly affect rVSV-F cytotoxicity against SCC 38 compared to viral therapy alone (At 72 hours, $P = .358$); however, cytotoxicity was slightly decreased in SCC 09 ($P = .016$).

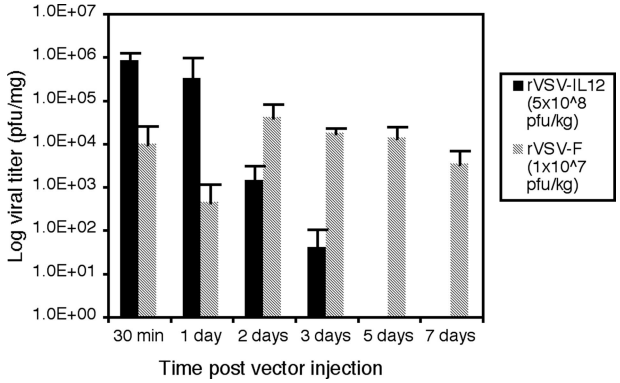

Fig. 4. Intratumoral replication of rVSV vectors in vivo. Replication of rVSV-IL12 and rVSV-F in floor of mouth squamous cell carcinoma (SCC) tumors at 30 minutes, 1 day, 2 days, 3 days, 5 days, and 7 days after intratumoral injection of vector. Tumor tissue was harvested for virus extraction and samples were analyzed by plaque assays. Error bars indicate standard deviation (SD).

volume compared to control animals receiving PBS (n = 9) or cisplatin alone (n = 7) ($P < .005$ for each virus at day 21) (Fig. 5). Combination rVSV-F + cisplatin decreased tumor size versus animals receiving rVSV-F alone, but did not reach significance (n = 9) ($P = .195$ at day 21). Conversely, monotherapy with rVSV-IL12 alone (n = 9) resulted in improved tumor control versus rVSV-IL12 + cisplatin, but not significantly ($P = .061$ at day 21). rVSV-IL12 monotherapy also yielded the greatest reduction in tumor size among all other groups ($P < .05$ vs. all other groups at day 21).

In Vivo Survival

Kaplan-Meier survival curves demonstrate that animals treated with rVSV-IL12 alone had better survival than control animals and all other treatment arms (Fig. 6). By day 25, none of the control PBS animals survived. At the same

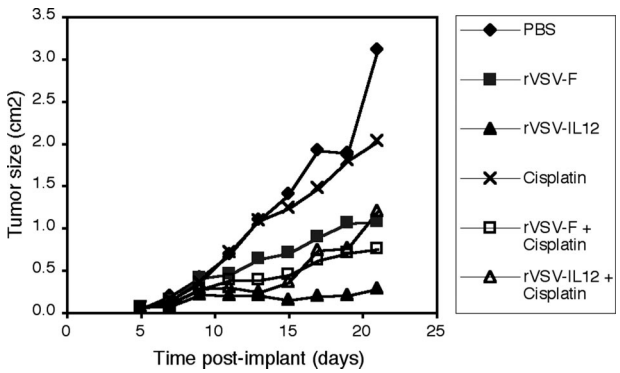

Fig. 5. Floor of mouth squamous cell carcinoma VII tumor area (greatest tumor height × width) after treatment with five daily intratumoral injections of rVSV-F (1×10^7 plaque-forming units), rVSV-IL12 (1×10^7 plaque-forming units), with or without five daily intraperitoneal injections of cisplatin (1.0 mg/kg) versus phosphate-buffered saline. Mice treated with rVSV-IL12 alone had markedly smaller tumors than all other treatment arms at day 21 ($P < .02$). Addition of cisplatin to rVSV-IL12 did not improve control of tumor size, whereas cisplatin with rVSV-F reduced tumor size at day 21 compared to virus alone ($P = .002$).

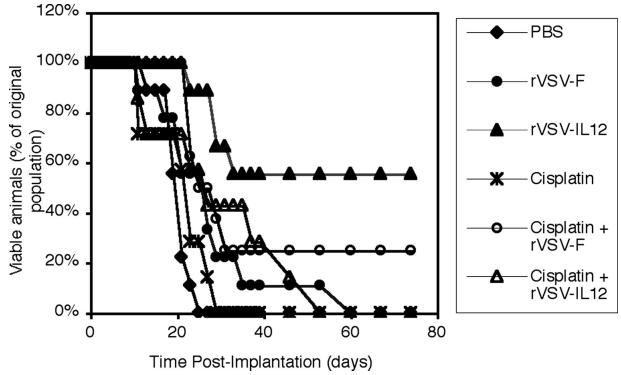

Fig. 6. Animal survival. Kaplan-Meier survival curves of mice with floor of mouth squamous cell carcinoma VII tumors after treatment with five daily intratumoral injections of rVSV-F (1×10^7 plaque-forming units) or rVSV-IL12 (1×10^7 plaque-forming units) starting on day 5 postimplantation of tumor cells, with or without five daily intraperitoneal injections of cisplatin (1.0 mg/kg) starting on day 6 postimplantation versus phosphate-buffered saline control (control n = 9, rVSV-F n = 9, rVSV-IL12 n = 9, rVSV-F + cisplatin n = 8, rVSV-IL12 + cisplatin n = 7). By day 60, only animals treated with rVSV-IL12 and rVSV-F + cisplatin survived. The rVSV-IL12 treated animals had significantly improved survival over combination therapy rVSV-IL12 + cisplatin animals ($P = .024$). Combination rVSV-F + cisplatin increased survival over rVSV-F alone, but did not reach significance ($P = .234$).

time point, 89% of the rVSV-IL12 group survived, while the combination virus and chemotherapy groups had survival of 57%, and 50% survival for rVSV-IL12 and rVSV-F, respectively. At 60 days, only the rVSV-IL12 monotherapy group (56%) and the rVSV-F + cisplatin group (25%) had animals surviving. These animals were alive and tumor-free at the last measurement (day 95).

No significant benefit was shown with the addition of cisplatin chemotherapy to the viral vector therapy. The difference in the survival curves between the rVSV-IL12 group and the rVSV-IL12 + cisplatin group was highly significant ($P = .024$), in favor of rVSV-IL12 alone. However, addition of cisplatin to rVSV-F showed a trend toward improved survival ($P = .234$) versus virus alone.

DISCUSSION

Conditionally replicating viruses targeted to tumors are being developed as a novel class of oncolytic agents. Vesicular stomatitis virus is a negative-strand RNA virus with inherent specificity for replication in tumor cells due to the attenuated antiviral response in these cells. VSV is a potent oncolytic virus as demonstrated in several preclinical tumor models, including glioma, hepatocellular carcinoma, breast carcinoma, and melanoma.

Ebert et al. generated a fusogenic VSV with enhanced oncolytic potential for the treatment of hepatocellular carcinoma.[6] The sequence coding for a mutated Newcastle disease viral protein, NDV-F(L289A), was incorporated into VSV to form rVSV-NDV/F(L289A). The viral vector was selectively infective of tumor cells and was able to induce tumor cell membrane fusion, resulting in large multinucleated syncytia. Syncytia formation led to subsequent cell death and may have triggered highly efficient immune activation, enhancing the cytopathic effects of VSV.

Interleukin 12 (IL12) is a cytokine released from activated antigen-presenting cells which has been shown to inhibit tumor growth in animal models through its ability to enhance the activity of cytotoxic T lymphocytes, induce interferon-gamma (IFN-γ) production by natural killer cells (NK), and inhibit angiogenesis by inducing IP-10 production from activated monocytes.[11] Mice bearing syngeneic metastatic colon cancer to the liver were effectively treated with intratumoral injection of an adenovirus vector expressing the murine IL12 gene (Adv.mIL12).[9] No systemic or organ toxicities were observed at the effective doses of Adv.mIL12 in tumor-bearing mice.[12]

We previously demonstrated recombinant VSV vectors incorporating viral fusion protein (rVSV-F) and interleukin 12 (rVSV-IL12) to have significant antitumor effects against squamous cell carcinoma (SCC) in an orthotopic murine model.[4,5] After multiple doses of the vector were injected intratumorally, rVSV-F and rVSV-IL12 effectively reduced tumor burden without apparent host toxicity, which led to prolongation of animal survival.

Combination therapy using multiple antineoplastic agents is an attractive approach to cancer therapy. It is theorized that multimodality therapy could potentiate the antitumor efficacy of each agent, while minimizing the toxicity by reducing the required doses for tumorlytic activity. In addition, using combinations of therapies that act through different mechanisms may discourage the emergence of treatment-resistant disease. In previous studies, IL12 in combination with cisplatin was shown to have stronger antitumor activity than either agent alone.[13] Since cisplatin is considered the most effective chemotherapeutic agent against SCC of the head and neck, it seemed the logical choice for trial with IL12 producing virus.[6] In a meta-analysis by Bourhis et al.,[6] platinum-based regimens were the most effective of the chemotherapy regimens ($P < .00001$). Furthermore, cisplatin has been shown to cause tumor regression in combination with oncolytic adenovirus ONYX-015 in a phase II trial and prolonged animal survival in a human head and neck xenograft tumor model.[3,14]

The aim of this study was to expand on the previous experiments with rVSV-F and rVSV-IL12 and to improve the 40% cure rate achieved by rVSV-IL12 alone by the addition of cisplatin chemotherapy as treatment for murine squamous cell carcinoma of the head and neck. The in vitro concentration of cisplatin was chosen as an approximation of plasma cisplatin levels 30 minutes following a 6 mg/kg intraperitoneal injection.[15] Originally, a 4 mg/kg IP daily dose of cisplatin was administered for 5 consecutive days in vivo as in the ONYX-015 trials by Heise et al.[14]; however, this dose was overly toxic when used in combination with recombinant VSV and was decreased to 1 mg/kg for these trials (data not shown). In vitro, both viruses demonstrated rapid replication with greater than 1,000-fold increase in titers over 48 hours with or without cisplatin. Viral infection in combination with cisplatin resulted in substantial death of SCC lines compared to human keratinocytes at 72 hours and improved the cytotoxicity for both viruses. Cisplatin did not hinder the ability of either virus to replicate in or kill murine SCC VII or human SCC 38 cells in vitro (Figs. 1–3). Combination therapy did not alter the preferential killing of murine or human SCC cells when compared to human keratinocytes (Figs. 2–3).

Plaque assays after single intratumoral injections of viral vectors showed an increase of viral titers for rVSV-F from day 1 to day 2, while rVSV-IL12 titers gradually decreased to undetectable levels by day 5 (Fig. 4). The results demonstrate the intratumoral replication of rVSV-F, which is likely responsible for the tumor destruction, improved tumor control, and survival prolongation previously reported by Shin et al.[5] Intratumoral replication of rVSV-IL12 was possibly attenuated by the emergence of T-cell-independent neutralizing IgM antibodies.[16] The in vivo replication advantage of rVSV-F over rVSV-IL12 is expected due to its additional mechanism of propagation via cell membrane fusion and giant cell formation. These findings suggest that the major effect of rVSV-IL12 is due to the production of IL12 and the subsequent host immune response.

Results of in vivo combination therapy on tumor size and survival were mixed (Figs. 5–6). In our SCC VII orthotopic floor of mouth model, addition of cisplatin to rVSV-F tended to reduce tumor volume ($P = .195$) and prolong animal survival ($P = .234$) when compared to monotherapy with rVSV-F. Treatment with rVSV-IL12 alone, however, was better at reducing tumor volume ($P = .061$ at 21 days) and significantly prolonged survival ($P = .024$) compared to combination therapy with cisplatin. In fact, rVSV-IL12 monotherapy yielded statistically better results than all other treatment arms for tumor size and survival, with 56% of animals alive and tumor-free at day 95. These results were somewhat unexpected in light of results from our in vitro studies and previous studies by Lamont et al.[3] and Heise et al.[14] In vitro, we found combination therapy with rVSV-IL12 + cisplatin was more cytotoxic to tumor cells and did not increase toxicity to human keratinocytes compared to monotherapy. These results, however, did not translate to improved efficacy in vivo.

Given that rVSV-IL12 is able to reduce tumor burden and prolong animal survival and that the major effect is probably due to IL12, it is likely that cisplatin inhibits IL12 mediated effects through one or more possible mechanisms. One explanation is that cisplatin could hinder the immune cell (NK or lymphocytic) response to IL12 due to host toxicity—creating an immunocompromised state. Cisplatin could also decrease cellular production of IL12 in infected cells. Alternatively, the combination of rVSV-IL12 and cisplatin may be significantly more toxic in vivo to normal murine cells or organ systems than was shown in vitro with normal human keratinocytes.

Although the experiments in this study showed in vivo enhancement of oncolytic virus therapy by chemotherapy in only one of two model systems, future investigations of combination therapy may still be warranted. Combination IL-12 gene therapy and cyclophosphamide has been shown to inhibit tumor growth and prolong animal survival in a murine melanoma model.[17] Doxorubicin was found to be more effective than cisplatin in combination therapy with IL-12 in a murine leukemia model.[18] Further studies of rVSV-F and rVSV-IL12 with cyclophosphamide, doxorubicin, or other chemotherapeutic agents may improve efficacy over cisplatin due to their different

mechanisms of action. In addition, second and third generation oncolytic VSVs are currently in development that are designed to increase viral infectivity while decreasing host toxicity. Future studies may incorporate the new oncolytic viral agents with different chemotherapeutic agents to search for synergistic antitumor effects for improved overall survival. Furthermore, considering the role of NK cells and T cells in the action of rVSV-IL12, future studies characterizing the immune effector cells in our model as well as involving immune modulators such as granulocyte-macrophage colony-stimulating-factor (GM-CSF) or anti-4-1BB antibody, which acts upon the T lymphocyte costimulator 4-1BB, may be warranted.[19]

CONCLUSION

Combination recombinant vesicular stomatitis virus with cisplatin chemotherapy effectively replicated and killed murine and human squamous cell carcinoma cells in vitro. Addition of cisplatin did not affect viral replication and improved cytotoxicity in murine SCC. In vivo, combination therapy enhanced rVSV-F antitumor activity, but diminished rVSV-IL12 antitumor activity. The combined modality strategy may provide useful treatment alternatives for head and neck squamous cell carcinoma with the development of more efficient viral vectors in combination with different chemotherapy agents or immunostimulatory agents.

Acknowledgments

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