Isolated Limb perfusion of oncolytic vesicular stomatitis virus to extremity sarcoma in immune-competent rats

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INTRODUCTION

Oncolytic virus provides an attractive new tool for treatment of solid tumors because of their abilities to replicate selectively within the tumor and kill neighboring cancer cells upon tumor lysis. Vesicular stomatitis virus (VSV) is a non-pathogenic RNA virus that is extremely sensitive to the antiviral actions of interferon (IFN) in normal cells but not in cancer cells. It has been postulated that this finding is due to the fact that IFN-responsive antiviral pathways are defective in many types of tumors. VSV is particularly appealing because of its rapid replication rate of 8 to 12 hours in tumor cells, such that significant tumor destruction may have occurred before the initiation of potentially neutralizing antiviral immune responses in the host.

Isolated limb perfusion (ILP) is a treatment modality for malignancies of the extremities in which the tumor-bearing limb is isolated from the patient’s circulation system and perfused separately. This procedure allows the administration of anti-cancer agents to the tumor at high doses with only a minimum risk of systemic toxicity. High-dose regional chemotherapy was one of the first concepts in ILP, which was complemented recently by the application of cytokines and immune-modulators frequently in combination with hyperthermia. More recently ILP was explored as a gene delivery system to extremity sarcoma.

The purpose of this study is to evaluate oncolytic activity of VSV in human osteosarcoma cell lines and the potential of ILP for efficient oncolytic virus transfer to extremity sarcoma in immune-competent rats.

MATERIALS AND METHODS

Cell culture. Two human osteosarcoma cell lines, Saos-2 and MG-63, were purchased from the American Type Culture Collection (Manassas, VA). Rat osteosarcoma cell line MSK was purchased from the Japan Health Sciences Foundation (Tokyo, Japan). Two human bone marrow stromal cells (MSC605 and MSC701) were isolated from bone marrow aspirates of normal human donors.

Virus generation and replication assays. To evaluate viral replication in osteosarcoma cells in vitro, 2 X 10^6 cells/well were seeded in 6-well plates overnight and then infected with recombinant VSV encoding the lacZ gene (rVSV-lacZ) at various MOI (1 – 10^5). At 24 hours later cells were stained in X-gal staining solution (Invitrogen, Carlsbad, CA). A sample of cell culture supernatant was collected at designated time points after infection and assayed for viral RNA genome by real-time RT-PCR using specific primers.

Cytotoxicity assay. Subconfluent cells harvested by trypsinization were plated in 24–well plates (5 X 10^4 cells/well) and allowed to adhere overnight. Cells were infected with VSV at various MOI (1 – 10^-2). The viable cell amount was measured by Cell Counting Kit-8 (Dojin, Kumamoto, Japan), according to the instructions. Cell viability was expressed as a percentage of the absorbance of treated cells vs. untreated cells.

In vivo study. Xenografts of rat osteosarcoma MSK were initiated by subcutaneous injections of 1 X 10^5 cells into the right hind limb just above the ankle of male Fisher 344 rat at the age of 6 weeks (CLEA, Tokyo, Japan). One week later, ILP was performed in rats by cannulating the femoral artery and vein, isolating the tumor-bearing hind limb from systemic circulation by tourniquet, and cycling perfusate including VSV (1.3 x 10^7 pfu) for 15 min at a rate of 2 ml/min. The smallest and largest diameters of tumors, and the body weights were measured once a week. All animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee and the protocol was approved by the Ethics Committee for Experimental Animals of Hiroshima University.

Histology and immunohistochemical staining. Two sections each of perfused tumor, perfused leg muscle, nonperfused muscle, brain, liver, and lung were obtained 48 h after VSV infusion. Samples were fixed in 4% parafformaldehyde overnight, and paraffin-embedded. Thin section (5 µm) was subjected to either hematoxylin and eosin (H&E) staining or immunohistochemistry using a monoclonal antibody against the VSV-G protein (VSV11-M, Alpha Diagnostic, San Antonio, TX). Immunohistochemistry sections were counterstained with hematoxylin.

RESULTS

Oncolytic activities of VSV in osteosarcoma cells. To assess whether VSV has the ability to preferentially replicate in osteosarcoma cells, the human and rat osteosarcoma cell lines as well as primary bone marrow stromal cells were examined in virus infection assays. VSV is fully capable of replicating its RNA genome in all osteosarcoma cell lines, but its ability to do so is severely attenuated in normal bone marrow stromal cells. Additionally, only osteosarcoma cells transduced with rVSV-lacZ at an MOI 0.001 uniformly expressed beta-gal.

In vivo anti-tumor efficacy and tumor-selective replication of VSV after ILP. All rats survived without significant differences of body weights between the VSV-treated group and the control group. MSK xenograft sarcoma in the control group grew rapidly in rats. In contrast, VSV delivered by ILP inhibited sarcoma growth. Four weeks after ILP, the mean tumor volume was significantly smaller than that of the control group (p < 0.01, Student’s t-test) (Fig. 1).

DISCUSSION

We demonstrate efficient VSV gene expression and replication in human osteosarcoma cells. In contrast, normal human bone marrow stromal cells are refractory not only for virus replication but also for viral gene expression. Defects in the IFN signaling pathway in several tumor cell lines have been shown to be responsible for tumor-specific VSV replication and cell lysis. Our findings suggest that osteosarcoma cells may have an intrinsically poor ability to produce and respond to IFN, like many cancer cells.

Our in vivo study also show that ILP of VSV into osteosarcoma tumors has potent antitumor activity and tumor selectivity, suggesting that VSV may be an attractive oncolytic agent against osteosarcoma in immune-competent hosts and novel VSV-mediated virotherapy for extremity sarcoma could be efficiently delivered by ILP.

Fig. 1. Anti-tumor effects of VSV delivered by ILP on extremity sarcoma growth in immune-competent rats. ** p < 0.01 as determined by Student’s t-test.