Gynecologic Oncology 132 (2014) 194–202

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Oncolytic measles and vesicular stomatitis virotherapy for endometrial cancer

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HIGHLIGHTS

- Endometrial cancer (EC) cell lines and xenografts undergo oncolysis when exposed to measles virus (MV) and vesicular stomatitis virus (VSV).
- VSV is more potent in EC oncolysis than MV.
- A phase 1 clinical trial of VSV in EC is warranted.

ARTICLE INFO

Article history:
Received 2 September 2013
Accepted 8 November 2013
Available online 15 November 2013

Keywords:
Endometrial cancer
Oncolytic virotherapy
Vesicular stomatitis virus
Measles virus

ABSTRACT

Objective. Current adjuvant therapy for advanced-stage, recurrent, and high-risk endometrial cancer (EC) has not reduced mortality from this malignancy, and novel systemic therapies are imperative. Oncolytic viral therapy has been shown to be effective in the treatment of gynecologic cancers, and we investigated the in vitro and in vivo efficacy of the Edmonston strain of measles virus (MV) and vesicular stomatitis virus (VSV) on EC.

Methods. Human EC cell lines (HEC-1-A, Ishikawa, KLE, RL95-2, AN3 CA, ARK-1, ARK-2, and SPEC-2) were infected with Edmonston strain MV expressing the thyroidal sodium iodide symporter, VSV expressing either human or murine IFN-β, or recombinant VSV with a methionine deletion at residue 51 of the matrix protein and expressing the sodium iodide symporter. Xenografts of HEC-1-A and AN3 CA generated in athymic mice were treated with intratumoral MV or VSV or intravenous VSV.

Results. In vitro, all cell lines were susceptible to infection and cell killing by all 3 VSV strains except KLE. In addition, the majority of EC cell lines were defective in their ability to respond to type I IFN. Intratumoral VSV-treated tumors regressed more rapidly than MV-treated tumors, and intravenous VSV resulted in effective tumor control in 100% of mice. Survival was significantly longer for mice treated with any of the 3 VSV strains compared with saline.

Conclusion. VSV is clearly more potent in EC oncolysis than MV. A phase 1 clinical trial of VSV in EC is warranted.

Introduction

While low-risk stage I endometrial cancer (EC) has a 5-year overall survival of 96% with surgical extirpation alone, 5-year overall survival for stage III and IV disease is 67% and 16%, respectively [1]. In addition, optimal therapy for high-risk early-stage and advanced-stage disease remains unclear. External beam radiotherapy [2–4], systemic chemotherapy [5,6], combined chemotherapy and radiation [7,8], and most recently, biologics [9,10] have been and continue to be investigated as adjuvant therapies for EC after surgical staging. However, despite nearly 3 decades of randomized controlled trials of adjuvant therapy, 5-year overall survival in metastatic EC continues to decline [1,11].

Abbreviations: CD46, cluster of differentiation 46; DMEM, Dulbecco Modified Eagle Medium; EC, endometrial cancer; GFP, green fluorescent protein; hIFNβ, human IFN-β; IT, intratumoral; IV, intravenous; LDLR, low density lipoprotein receptor; M51 protein, residue 51 of the matrix protein; mIFNβ, murine IFN-β; MOI, multiplicity of infection; MV, measles virus; MV-NIS, Edmonston strain MV expressing the thyroidal sodium iodide symporter; NIS, sodium iodide symporter; NOAEL, no adverse event level; OV, oncolytic virus; PE, R-phycoerythrin; PVRL4, poliovirus receptor-related 4; qRT, quantitative real-time; $TCID_{50}$, 50% tissue culture infective dose; VSV, vesicular stomatitis virus; VSV-hIFNβ, vesicular stomatitis virus expressing human IFN-β; VSV-mIFNβ, vesicular stomatitis virus expressing murine IFN-β; VSV-M51-NIS, vesicular stomatitis virus with a methionine deletion at residue 51 of the matrix protein and expressing the sodium iodide symporter.

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http://dx.doi.org/10.1016/j.ygyno.2013.11.010

While isolated EC vaginal recurrences can be treated with pelvic radiation and brachytherapy [12], multisite and distant recurrence often portends death from disease [1]. Numerous systemic cytotoxic therapies have been investigated for recurrent EC [13–17], with response rates ranging from 0% for oral etoposide [17] to 27.3% for single-agent paclitaxel [15]. However, the response rate for most single-agent chemotherapies investigated in patients with recurrent disease remains in the single-digit percentages [14,16]. Additionally, in the past decade, biologic agents have emerged, and their activity as single agents in the patients with recurrent EC has been similar to that of cytotoxic agents, with response rates ranging from 3.3% for lapatinib [18] to 13.5% for bevacizumab [9]. And while progestin therapy for recurrent EC may extend progression-free survival, it does not improve overall survival [19].

Taken together, the substantial risk of recurrence in advanced-stage EC and the modest response to systemic therapies in patients with recurrence suggest that novel approaches are imperative in mitigating the high risk of death from advanced-stage and recurrent EC.

It has been a century since the first published account of viral oncolytic activity in gynecologic cancer when an advanced cervical cancer regressed in response to rabies vaccination [20]. Early clinical trials used wild-type viruses; however, bioengineering of oncolytic viruses (OVs) has reenergized them as potentially potent therapies for malignancy. Ovarian cancer is the gynecologic malignancy that has garnered the most OV therapy focus. Measles virus (MV), vaccinia virus, vesicular stomatitis virus (VSV), herpes simplex virus, reovirus, and several adenoviruses all have shown activity against ovarian cancer in preclinical cancer models [21–25], and ovarian cancer clinical trials have been performed using MV, herpes simplex virus, reovirus, and adenoviruses [24,26–28]. However, OV activity in other gynecologic cancers is not well studied, and to our knowledge, only 3 cases of EC treated with an OV have been published. This small cohort treated on a phase 1 adenoviral trial had promising results, with 2 recurrent ECs maintaining stable disease [27].

A highly promising OV, VSV is a negative-stranded RNA virus of the Rhabdoviridae family, which is nonpathogenic in humans and has recently entered clinical testing with human IFN-β (VSV-hIFNβ) for patients with hepatocellular carcinoma at Mayo Clinic in Arizona. Mucous membrane lesions develop in the mouth and nose of infected domestic and farm animals and infection is nonlethal. VSV infects cells through the low density lipid receptor (LDLR) and replicates in the cytoplasm without integrating the viral genome into the host genome [29]. The VSV genome can be manipulated to insert and express transgenes, but it does not have transforming activity [30]. VSV infection initiates a cascade that begins with innate immune system activation and IFN-β production [31]. IFN-β activates genes, upregulates antigen processing machinery, and activates antigen-presenting cells, which stimulates leukocytes to clear the infected cells [32]. Malignant cells have dysfunctional translational and defective immune signaling. And while the normal cellular response to viral infection involves cessation of translation through IFN-dependent pathways, malignant cells have defective IFN pathways, allowing high levels of viral loads that lead to cell death [33]. Additionally, engineering VSV to code for IFN-β attenuates the effect of the virus by inducing an antiviral state in normal cells surrounding malignant cells [34]. VSV antitumor activity has been demonstrated in cancers of the ovary [23], prostate [35], head and neck [36], and colon [37], melanoma [38], and hepatocellular carcinoma [39].

Given the high mortality associated with advanced-stage and recurrent EC, efficacy limitations of currently available therapies, and promising antitumor potency of OVs, we elected to investigate their activity in EC. We present preclinical efficacy of recombinant Edmonston strain MV expressing the thyroidal sodium iodide symporter (MV-NIS) and recombinant VSV expressing either human IFN-β (VSV-hIFNβ) or murine IFN-β (VSV-mIFNβ) in EC. To our knowledge, this is the first published report of MV and VSV activity in EC.

Methods

Cells and viruses

Type I human EC cell lines HEC-1-A, Ishikawa, KLE, RL95-2, and AN3 CA and type II cell lines ARK-1, ARK-2, and SPEC-2 cells were used. KLE and RL95-2 were cultured in Dulbecco Modified Eagle Medium (DMEM) and Ham F-12 Nutrient Mixture (DMEM/F12; Mediatech, Herndon, Virginia) supplemented with 10% FBS (Life Technologies, Grand Island, New York). AN3 CA and Ishikawa were cultured in 10% FBS DMEM (Mediatech). HEC-1-A, ARK-1, ARK-2, and SPEC-2 were maintained in 10% FBS RPMI-1640 (Mediatech). African green monkey kidney Vero cells (CCL-81; American Type Culture Collection, Manassas, Virginia) were maintained in 5% FBS DMEM.

Expression levels of MV and VSV receptors on all EC cell lines were determined by flow cytometry using R-phycoerythrin (PE)-conjugated antibodies specifically against the MV receptors CD46 (624048, BD, Franklin Lakes, NJ) and PVRL4 (FAB2659P, R&D), and the VSV receptor, LDLR (FAB2148P, R&D).

The following viruses were propagated as previously described: Edmonston strain MV expressing the thyroidal sodium iodide symporter (MV-NIS) [40,41], VSV expressing hIFNβ (VSV-hIFNβ) or mIFNβ (VSV-mIFNβ) [42] and VSV expressing NIS and with a methionine deletion at residue 51 of the matrix protein (VSV-M51-NIS), which abolishes the functions of the M protein to block the nuclear to cytoplasmic transport of IFN-β mRNA [43]. Viral titers were determined by 50% tissue culture infective dose ($TCID_{50}$) assay on Vero cells.

Virus infection, cell viability, and progeny production

For the virus infection assays, cells in 96 well plates were exposed to MV-NIS, VSV-hIFNβ, VSV-mIFNβ, or VSV-M51-NIS at multiplicity of infection (MOI; 0, 0.001, 0.01, 0.1, 1, and 10). Cell viability was assessed at 48 h (VSV) or 120 h (MV) post infection using the MTS cell proliferation assay according to manufacturer's instructions (Promega, Madison, WI).

For viral progeny propagation assays, cells (2×10^5/well) were seeded in 6 well plates and infected with viruses (MOI 0.02). Two hours later, the virus inoculum was removed, cells were washed with PBS and growth media were replaced. Media (for VSV) or cells (for MV) were collected at the indicated time points. Viral titers were determined by $TCID_{50}$ assay on Vero cells.

Sensitivity of ECs to IFN

To evaluate whether EC cells have a functional IFN antiviral response pathway, the panel of EC cell lines in 96 well plates was incubated overnight with increasing concentrations of human IFN-α (Universal Type I Interferon; R&D, Piscataway, New Jersey). The next day, VSV expressing green fluorescent protein (VSV-GFP) (MOI 0.1 or 1.0) was added to the cells. Cell viability was determined by the cell proliferation MTS assay 48 h later.

Animal experiments

All animal experiments were approved by and performed according to the guidelines of the Mayo Clinic Institutional Animal Care and Use Committee. Four- to 5-week-old female athymic mice were purchased from Harlan (Indianapolis, Indiana). Mice were implanted subcutaneously in the right flank with 2×10^6 HEC-1-A or AN3 CA cells. When tumors reached 0.3 to 0.5 cm in diameter, 100 μL of virus or saline was injected intratumorally (10^7 $TCID_{50}$/mouse) or intravenously through the tail vein (10^6 $TCID_{50}$/mouse). Of note, IV MV-NIS was not evaluated in these preclinical studies as immunocompetent humans vaccinated against MV have neutralizing antibodies that rapidly inactivate the virus and, as such, IV MV human clinical trials will not be planned. Tumors were measured twice per week, and mice were euthanized