Dual Therapy of Ovarian Cancer Using Measles Viruses Expressing Carcinoembryonic Antigen and Sodium Iodide Symporter

Kosei Hasegawa, Linh Pham, Michael K. O’Connor, Mark J. Federspiel, Stephen J. Russell, and Kah-Whye Peng

Abstract

Purpose: MV-CEA is an oncolytic measles virus currently being tested in patients with ovarian cancer and whose propagation can be monitored by measuring blood carcinoembryonic antigen (CEA) levels. MV-NIS is an oncolytic measles virus coding for the thyroidal sodium iodide symporter (NIS) whose propagation can be mapped by serial radioiodine imaging. Expression of both CEA and NIS genes from a single virus would combine sensitive, quantitative expression monitoring (CEA) with radioisotopic expression mapping (NIS). Because of the unfavorable replication kinetics of measles viruses expressing both CEA and NIS, we explored the feasibility of combining MV-CEA with MV-NIS for comprehensive virotherapy monitoring in ovarian cancer.

Experimental Design and Results: Mice implanted with i.p. SKOV3ip.1 ovarian cancer xenografts received MV-CEA alone, MV-NIS alone, or a combination of MV-CEA plus MV-NIS. Viral gene expression was monitored by measuring blood CEA levels, and the location of virus-infected cells was monitored by gamma camera imaging. Surprisingly, mice receiving the combination of MV-CEA plus MV-NIS showed greatly superior responses to therapy, but this was associated with 10-fold lower plasma levels of CEA compared with mice treated with MV-CEA alone. In vitro studies showed superior replication kinetics of MV-NIS relative to MV-CEA. The gamma camera scans were considerably less sensitive than the plasma CEA marker for monitoring virus infection.

Conclusions: Dual therapy with MV-CEA and MV-NIS is superior to treatment with either virus alone, and it allows noninvasive monitoring of virotherapy via soluble marker peptide and gamma camera imaging. This has important implications for the clinical development of oncolytic measles viruses.

Much progress has been made in the development and use of replication-competent oncolytic viruses for cancer therapy (1, 2). Oncolytic viruses, such as conditionally replicating adenoviruses, herpes simplex virus, reovirus, echovirus type 1, and attenuated mumps virus, have shown promise in preclinical models of ovarian cancer and some are being evaluated in clinical trials (3–11). We have been developing the live attenuated Edmonston B measles virus for cancer therapy and shown that the virus has potent antitumor activity for a variety of human malignancies (12–16). The virus selectively causes extensive intercellular fusion in tumor cells but minimal cytopathic damage in normal cells (13, 14). The mechanistic basis for this selective tumor destruction remains to be completely elucidated but is in part due to high levels of CD46, the receptor required for virus entry and cell fusion, expressed on surfaces of tumor cells compared with normal cells (17, 18). Oncolytic measles virus is able to discriminate between high and low surface densities of CD46, preferentially causing extensive cytotoxic damage of intercellular fusion in high CD46-expressing cells (19).

Ideally, oncolytic viruses for cancer therapy should be genetically modified to enable noninvasive monitoring of the profiles and sites of virus propagation in the treated patient. Virus pharmacokinetics and pharmacodynamics data would greatly facilitate analysis of clinical trial results and determine if a failed response to therapy is due to lack of virus infection, inadequate virus spread, weakened cytotoxic effect, or premature virus elimination. The most common modality for monitoring viral gene expression is radionuclide-based gamma camera imaging or positron emission tomography. Marker proteins, such as herpes simplex virus-1 thymidine kinase, dopamine receptor, somatostatin receptor, or human thyroidal sodium iodide symporter (NIS) concentrate radionuclides in the infected cells to allow precise mapping of sites of gene expression (20–23). Subsequent dosimetry calculations from gamma camera or positron emission tomography images enable quantitation of relative radioisotope uptake at the predominant sites of gene expression (24). On the other hand, use of soluble marker peptides, such as the extracellular domain of human carcinoembryonic antigen (CEA) or β-chain of...
human chorionic gonadotropin (hCG), provides a highly sensitive and quantitative way for regular monitoring of virotherapy to guide dose-escalation studies (15, 25, 26). In contrast to gamma camera or positron emission tomography imaging, monitoring using soluble marker peptide is much less costly and more amenable to intensive monitoring through regular blood draws or urine samples.

Oncolytic measles viruses expressing NIS or CEA have been generated (Fig. 1). The transgenes were inserted as independent transcription units flanked by MluI and AatII restriction sites into the first (MV-CEA) or fifth position (MV-NIS) of the full-length infectious clone of Edmonston measles virus (12, 25). These viruses have promising antitumor activity against a variety of human tumor xenografts in mice (12, 14, 15). MV-CEA virus is currently being tested in a phase I dose-escalation trial [10^3 to 10^9 plaque-forming unit (pfu)] for i.p. delivery into patients with recurrent ovarian cancer (14). MV-NIS virus is currently undergoing toxicology testing and regulatory approval for i.v. administration into patients with relapsed multiple myeloma (12). Concentration of β-emitting radionuclides, such as ^131I, by MV-NIS-infected cells, also offers the opportunity to potentially enhance therapeutic outcome by combining virotherapy with radiation therapy (12, 27, 28). To combine the sensitivity of CEA monitoring with radionuclide mapping of sites of gene expression, we have inserted both transgenes into the measles genome (29). However, the MV-NIS-CEA virus had poor replication kinetics and the maximal titer was 10-fold less than either parental viruses. Hence, instead of using a single measles virus with dual-monitoring transgenes, we wanted to evaluate the feasibility of combining both MV-CEA and MV-NIS for comprehensive virotherapy monitoring and test the therapy in an orthotopic murine model of human ovarian cancer.

Materials and Methods

Cell lines and viruses. The human epithelial ovarian cancer cell lines, SKOV3ip.1 and IGROV1, and low-passage primary cell lines established at the Mayo Clinic, OV202, were maintained in α-MEM (Irvine Scientific, Irvine, CA) supplemented with 20% fetal bovine serum and 2 nmol/L glutamine (Invitrogen, Carlsbad, CA). African green monkey Vero cells (American Type Culture Collection, Manassas, VA) used for propagation of MV-CEA and MV-NIS viruses were maintained in 5% fetal bovine serum-DMEM (Invitrogen). Normal human dermal fibroblasts (NHDF) were purchased from Cambrex Bioscience (Walkersville, MD) and maintained in medium as recommended by the manufacturer (BioWhittaker, Inc., Walkersville, MD). Whole heparinized blood from healthy volunteers were centrifuged through a Ficoll Plus (GE Healthcare, Piscataway, NJ) gradient to obtain peripheral blood lymphocytes. MV-CEA and MV-NIS viruses were propagated on Vero cells. Cells were inoculated with MV-CEA or MV-NIS at a multiplicity of infection (MOI) of 0.1 for 2 hours at 37°C. The cell monolayer was incubated at 37°C until 90% of cells were

---

**Fig. 1.** Schematic representation of full-length infectious clones of MV-CEA and MV-NIS. The CEA cDNA was inserted as an additional transcription unit upstream of the MV-nucleocapsid (N) gene using MluI and AatII restriction sites whereas the NIS cDNA was inserted downstream of the hemagglutinin (H) gene. P, phosphoprotein; M, matrix; F, fusion; L, polymerase.
recruited into syncytia. Cell supernatants were harvested over 3 to 4 days, treated with benzonase to digest nucleic acids, filtered through a 5 μm filter, and concentrated by diafiltration (500 K). Viral stocks were frozen at -80°C until use. Virus titers were determined by 50% endpoint dilution (TCID₅₀) assays on Vero cells.

**Generation of Fluc- and bhCG-expressing SKOV3ip.1 cells.** To enable noninvasive monitoring of i.p. tumor growth, SKOV3ip.1 cells were transduced with HIV-1-based lentiviral vectors expressing firefly luciferase (Fluc) and bhCG. The reporter genes were cloned into the vector plasmid pHRSIN-CSGW-dlNotI (kindly provided by Dr. Y. Ikeda, Mayo Clinic, Rochester, MN) as BamHI-NotI PCR fragments. Lentiviral vectors were generated by transient transfection using the calcium phosphate method of plasmids encoding the vesicular stomatitis virus, G envelope, gag-pol genes, and pHRSIN-bhCG or pHRSIN-Fluc into 293T cells. Conditioned medium containing viral vectors were harvested 48 hours posttransfection, filtered (0.45 μm), and frozen until use. SKOV3ip.1 cells (2 × 10⁶ per 24-well plate) were transduced using 500 μl viral supernatants, and expression of Fluc and bhCG were confirmed by measuring cellular luciferase activity (IVIS 200, Xenogen Corporation, Alameda, CA) and harvest of conditioned medium and assay for soluble bhCG (Mayo Central Clinical Laboratory), respectively.

**Virus infection and ¹²⁵I uptake studies.** To compare the growth kinetics of MV-NIS and MV-CEA, Vero producer cells were plated in six-well plates (2 × 10⁵ per well) overnight and the next day, infected with virus at a high MOI of 2.0 or at low MOI of 0.02. The virus inoculum was removed 2 hours later and cells were incubated in 1 ml standard medium at 37°C. At various time points postinfection, cells were scraped into the medium and frozen at -76°C until analysis. Before titration on Vero cells by TCID₅₀ titration, the viral harvest was frozen and thawed twice to release the cell-associated virions.

For infection assays, cells were plated overnight (1 × 10⁵-2 × 10⁵ per well) and incubated with MV-CEA or MV-NIS diluted in Opti-MEM at a MOI of 0.5. After 2 hours, the virus inoculum was removed and standard medium was replaced. At 2 days postinfection, the cells were processed as follows. Cells were fixed with 0.5% glutaraldehyde, stained with 2% crystal violet, and photographed. For determination of virus antineoplastic activity, cells were trypanized and the numbers of live harvested 48 hours posttransfection, filtered (0.45 μm), and frozen until use. SKOV3ip.1 cells (2 × 10⁶ per 24-well plate) were transduced using 500 μl viral supernatants, and expression of Fluc and bhCG were confirmed by measuring cellular luciferase activity (IVIS 200, Xenogen Corporation, Alameda, CA) and harvest of conditioned medium and assay for soluble bhCG (Mayo Central Clinical Laboratory), respectively.

**Fig. 3.** Antitumor activities of oncolytic measles viruses. A, athymic mice bearing s.c. SKOV3ip.1 tumors were treated with two intratumoral injections of saline or MV-NIS (1 × 10⁶ pfu) delivered 2 weeks apart. Points, mean tumor volumes (n = 10 per group); bars, SE. B, average plasma CEA levels in mice (n = 4-5 per time point) bearing i.p. SKOV3ip.1 tumors. Mice bearing i.p. SKOV3ip.1 tumors were treated two i.p. doses of saline, MV-CEA (1 × 10⁶ pfu), or MV-CEA + MV-NIS (1 × 10⁶ pfu each) delivered 2 weeks apart. Mice were bled 1 week after initial virus and weekly thereafter. Limit of detection of assay is 35 ng/mL. C. Kaplan-Meier survival curves of mice treated with saline, MV-CEA, or MV-CEA + MV-NIS.

**Fig. 4.** Gamma camera images of MV-CEA or MV-NIS + MV-CEA – treated mice in the s.c. xenograft model (A) and i.p. xenograft model (B). Mice were injected i.v. with 0.5 μCi ⁹⁹mTc sodium pertechnetate and imaged 1 hour later to minimize accumulation of ⁹⁹mTc in the bladder, mice were given 0.5 μl saline s.c. to help empty the bladder. Corresponding plasma CEA levels and tumor weights at necropsy of MV-NIS + MV-CEA – treated mice in the i.p. model. n.d., not detectable (limit of detection is 15 ng/mL).
cells were counted by trypan blue exclusion. For 125I uptake, mock-infected, MV-CEA- or MV-NIS-infected cells (in triplicates, 12-well plates) were washed once in warm (37°C) HBSS (Invitrogen), incubated with 800 μL of warm 10 mmol/L HEPES-HBSS buffer with or without 100 μL of 100 mmol/L potassium perchlorate (KClO4), which inhibits NIS uptake of iodine and 100 μL of 125I (100,000 cpm/100 μL). The cells were incubated at 37°C for 45 minutes after which medium were aspirated and cells washed with 1 mL cold HEPES-HBSS. The cells were lysed with 1 mL of 1 mol/L NaOH for 15 minutes and the amount of radioactive uptake by the cells quantitated using a HBSS. The cells were lysed with 1 mL of 1 mol/L NaOH for 15 minutes and the amount of radioactive uptake by the cells quantitated using a

![Gamma camera imaging and CEA monitoring.](image)

**Table 1.** Weights of peritoneal tumors (g) and terminal plasma CEA (ng/mL) levels in all surviving mice at necropsy

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Peritoneal tumor weight</th>
<th>Plasma [CEA]</th>
<th>CEA/g tumor</th>
<th>Theoretical plasma [CEA]</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV-CEA</td>
<td>730</td>
<td>242</td>
<td>0.33</td>
<td>627.80</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>720</td>
<td>630</td>
<td>0.88</td>
<td>619.20</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>703</td>
<td>1.26</td>
<td>481.60</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>870</td>
<td>1,190</td>
<td>1.37</td>
<td>748.20</td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>568</td>
<td>0.63</td>
<td>774.00</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>447</td>
<td>0.72</td>
<td>533.20</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>733.33</td>
<td>630.00</td>
<td>630.67</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>133.82</td>
<td>318.37</td>
<td>115.08</td>
<td>0.46</td>
</tr>
<tr>
<td>MV-NIS+ MV-CEA</td>
<td>160</td>
<td>&lt;5</td>
<td>0.03</td>
<td>137.60</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>1,100</td>
<td>122</td>
<td>0.11</td>
<td>946.00</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>&lt;5</td>
<td>0.05</td>
<td>94.60</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>18</td>
<td>0.13</td>
<td>120.40</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>31</td>
<td>0.13</td>
<td>206.40</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>89</td>
<td>0.32</td>
<td>240.80</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>&lt;5</td>
<td>0.07</td>
<td>60.20</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>&lt;5</td>
<td>0.01</td>
<td>318.20</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>390</td>
<td>142</td>
<td>0.36</td>
<td>335.40</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>21</td>
<td>0.07</td>
<td>266.60</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>317.00</td>
<td>44.30</td>
<td>272.62</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>295.97</td>
<td>52.88</td>
<td>99.35</td>
<td>0.17</td>
</tr>
</tbody>
</table>

NOTE: The level of plasma CEA was correlated with tumor weight (CEA/g tumor) and the theoretical plasma CEA levels were calculated by multiplying tumor weights by 0.86. The deviation of experimental CEA levels from the calculated theoretical value is tabulated.

**Results**

In vitro antineoplastic activity of MV-NIS and 125I uptake. Ovarian cancer cell lines SKOV3ip.1 and IGROV1; a low-passage Mayo patient–derived cell line, OV202; NHDF; and peripheral blood lymphocytes were incubated with MV-NIS or
MV-CEA (MOI 0.5) for 2 hours after which the virus was removed. By 48 hours after virus infection, the characteristic cytopathic effects of syncytial formation were apparent in the tumor cells, whereas there was minimal cytopathic damage in NHDF (Fig. 2A).

The percentage of live cells in the infected cultures was compared against the mock-infected controls in cell killing assays. The cytopathic effects translated to significant cell killing in MV-CEA- and MV-NIS-infected tumor cells, whereas cell viability remained high in NHDF and peripheral blood lymphocyte cells where there was minimal intercellular fusion (Fig. 2B).

The ability of MV-NIS-infected ovarian cancer cells to uptake radioiodine ($^{125}$I) was evaluated in vitro. As shown in Fig. 2C, MV-NIS-infected SKOV3ip.1, IGROV1, and OV202 cells trapped significant amounts of $^{125}$I compared with the MV-CEA-infected cells. This uptake of radioiodine was specifically inhibited in vitro by addition of potassium perchlorate in the medium. In contrast to the peripheral blood lymphocytes, there was high uptake of $^{125}$I in the infected NHDF cells (Fig. 2C).

In vivo antitumor activity of MV-NIS and MV-CEA. We first evaluated the oncolytic activity of MV-NIS for SKOV3ip.1 ovarian tumors in a s.c. xenograft model. Athymic mice bearing s.c. (0.5 cm in diameter) SKOV3ip.1 xenografts were injected intratumorally with one dose of $10^6$ pfu MV-NIS or saline ($n = 10$ per group). Tumor growth was monitored over time (Fig. 3A). MV-NIS significantly inhibited growth of big tumors and induced complete regression of smaller tumors in four mice. In contrast, tumors in the saline-treated controls continued growing, resulting in a significant difference in tumor growth over time between the treated and control groups. Mice treated with MV-NIS or saline were given Tc-99m sodium pertechnetate and imaged using a gamma camera. As shown in Fig. 4A, there was a detectable, albeit weak, signal in the s.c. SKOV3ip.1 tumors of MV-NIS-treated mice. Strong signals were detected in the bladder (excretion of the radionuclide) and organs that normally express NIS—salivary glands, thyroid, and stomach (30).

Ovarian cancer is typically localized in the peritoneal cavity and is thus amenable to i.p. delivery of the virus. Human SKOV3ip.1 cells were seeded into the peritoneal cavity of athymic mice to simulate ovarian cancer and 1 week later when there was significant tumor burden, mice were given saline, MV-CEA alone, or a cocktail of MV-CEA + MV-NIS. Viral gene expression in the mice was followed by measuring plasma levels of the virally encoded CEA 1 week after initial virus therapy and weekly thereafter. Mice are not susceptible to measles virus infection and, hence, CEA levels reflect virus propagation in the human tumors. Results revealed that increase in plasma CEA levels in the MV-CEA + MV-NIS–treated group was delayed by 1 week compared with the group that received MV-CEA only (Fig. 3B). Although CEA levels increased steadily over time with virus propagation in the tumors, maximal plasma CEA levels in the MV-NIS-treated group remained consistently 10-fold lower. Maximal average CEA level in MV-NIS-treated mice was $69 \pm 99$ ng/mL (mean $\pm$ SD, $n = 17$) compared with $664 \pm 488$ ng/mL ($n = 14$) in the MV-CEA group. CEA was undetectable in plasma of saline-treated mice (data not shown).

From the low CEA values, it would seem that MV-CEA + MV-NIS–treated mice were tumor free or had minimal disease.
group was 0.86 compared with 0.13 in the MV-NIS + MV-CEA–treated group, indicating that there was proportionately less MV-CEA virus in these peritoneal tumors. In the absence of MV-NIS, the theoretical [CEA]/g tumor in the combination group would be 272 ng CEA/g tumor. Instead, the actual experimental value was 44.3, a reduction of >80% if the mice also received MV-NIS virus.

All mice in the control group were euthanized by day 48 due to large-volume ascites and the median survival was 28 days (Fig. 3C). All three survival curves were statistically different from each other (P < 0.001). Treated mice rarely developed ascites. The median survival of the MV-CEA-alone group was 59 days compared with >70 days in the MV-NIS + MV-CEA–treated group (Fig. 3C). All surviving mice were euthanized at day 70 postimplantation of cells due to large s.c. injection site tumor in the mice. Control saline-treated mice did not survive long enough to have these injection-site tumors. Necropsy revealed that MV-CEA + MV-NIS–treated group had lower tumor burden (Table 1) and disease is localized mainly in the omentum (data not shown). In contrast, MV-CEA-treated mice had tumors under the diaphragm surrounding the hepatic blood supply and at the omentum (data not shown).

To determine if there was a difference in amount of tumor destruction by the viruses at the earlier time points after initial therapy, we did additional experiments to include the MV-NIS-alone group and monitored tumor burden using bioluminescent imaging and analysis of secreted tumor marker peptide, bhCG, in the plasma of mice. SKOV3ip.1 cells stably expressing Fluc and bhCG were generated by lentiviral transduction and were implanted i.p. in athymic mice. One week later, mice received MV-CEA alone, MV-NIS alone, or a combination of MV-CEA + MV-NIS. Two weeks after initial therapy, tumor growth was significantly inhibited to a comparable extent in the virus-treated groups (Fig. 5A). In contrast, tumors in control mice continued to grow (increase of 7-fold). Using bioluminescent imaging, we were able to differentiate between i.p. tumors (localized mainly in the greater omental region) and a s.c. injection site tumor that was starting to grow in these mice (Fig. 6). By 35 days after cell implantation, quantitation of photons released by i.p. Fluc tumors indicated that mice that received MV-CEA + MV-NIS had significantly reduced tumor burden (Fig. 5B). Analysis of i.p. tumor burden at necropsy at day 60 confirmed the imaging data (Fig. 5C).

**Replication kinetics of MV-NIS and MV-CEA.** Propagation kinetics of MV-NIS and MV-CEA were compared in Vero producer cells at a low MOI of 0.02. Small syncytia were first observed in MV-NIS-infected cells at 24 hours postinfection, which became larger by 36 hours (Fig. 7A). Almost all cells in the MV-NIS-infected culture had fused by 48 hours and most had lifted by 72 hours. Growth curves of virus propagation confirmed that MV-NIS propagated faster and reached higher titers than MV-CEA (Fig. 7B).

**Discussion**

MV-NIS and MV-CEA are being developed for clinical testing, respectively, in patients with relapsed multiple myeloma and ovarian cancer. We wanted to evaluate the feasibility of combining both viruses for comprehensive virotherapy of ovarian cancer to enable noninvasive monitoring of measles virus gene expression via the highly sensitive and quantitative measurement of plasma CEA levels and radioisotope mapping of measles virus–infected sites. Potential augmentation of therapeutic outcome through concentration of β-emitting 131I radioiodine in NIS-expressing cells is also an attractive possibility (12, 31). Results from this study established the antitumor efficacy of MV-NIS for the treatment of s.c. and i.p. ovarian cancer in athymic mice. Tumor-bearing mice treated with MV-NIS survived significantly longer than saline-treated controls, to a similar extent as mice treated with MV-CEA. Monitoring of viral gene expression via the highly sensitive CEA assay revealed intriguing pharmacokinetic data on measles virotherapy in treated mice. In mice that received dual therapy with MV-CEA + MV-NIS viruses, increase in plasma CEA was delayed and overall CEA levels were 10-fold lower than mice that received MV-CEA alone. Dual therapy also unexpectedly resulted in a significantly lower i.p. tumor burden in these mice.

The mechanism for the better therapeutic outcome in mice that received dual therapy remains to be elucidated. It is

![Image](https://example.com/image.png)

**Fig. 6.** Bioluminescent images of SKOV3-Fluc-bhCG tumors in the various treatment groups. Arrows, i.p. tumors; arrowheads, s.c. injection–site tumors.

![Image](https://example.com/image.png)

**Day 6**

MV-CEA + MV-NIS

MV-CEA

MV-NIS

Control

![Image](https://example.com/image.png)

**Day 35**

MV-CEA + MV-NIS

MV-CEA

MV-NIS

Control
unlikely that this difference was due to the mice receiving twice more virus ($10^6$ pfu MV-NIS + $10^6$ pfu MV-CEA) as there was no significant difference in survival of mice treated with $10^4$ to $10^7$ pfu MV-CEA. In addition, monitoring of early tumor response using soluble bhCG marker peptide indicated that decrease in tumor burden and inhibition of tumor growth by the viruses were comparable in all the three groups.

Both viruses share a similar vector backbone except that the cDNA for NIS was inserted downstream of H, whereas CEA was inserted upstream of N, at the first position as an additional transcription unit (Fig. 1). In vitro, MV-NIS replicated faster than MV-CEA and is likely due to placement of the respective transgenes in the measles virus genome. Measles virus is a negative-strand RNA virus with six genes encoding eight proteins, nucleoprotein (N), phosphoprotein (P)/V/C, matrix (M), fusion (F), hemagglutinin (H), and large polymerase (L). Viral genome replication starts at the 3' end and there is a transcription gradient down the genome due to transcription attenuation at every gene junction (32). This implies that the levels of measles virus mRNA decrease progressivemly with the distance of the genes from the 3' end of the genome. It was estimated that the ratio of N/P/M/F/H/L transcripts in a measles virus–infected HeLa cell culture was 68:23:16:14:12:1 with the amount of viral protein correlating with the gene order (32). Although insertion of CEA cDNA at position 1 ensured high CEA protein expression, which is important for high-sensitivity detection of this marker peptide in body fluids of patients, it also means that transcription of downstream viral genes and proteins would be attenuated, likely resulting in slightly delayed replication kinetics of MV-CEA.

When both viruses were injected i.p. into mice implanted 1 week earlier with SKOV3ip.1 tumor cells, tumor cell killing by the virus treatment groups were comparable. However, the amount of virus given, although significantly prolonging the survival of mice compared with the saline group, was insufficient to result in total eradication of tumor burden. Unfortunately, we were not able to fully evaluate the true survival of the treated mice as numerous mice had a significant injection tumor that resulted in termination of the experiment. It is also evident that virus in infected peritoneal tumors do not disseminate to these injection site tumors. No live virus was rescued from injection-site tumor-Vero cell overlays and these tumors stained negative for measles N mRNA by in situ hybridization (data not shown).

SKOV3ip.1 cells do not secrete CEA and murine tissues are not susceptible to MV-CEA. The profiles of plasma CEA in this model are a reflection of viral gene expression in the human tumors. Dual therapy with MV-NIS virus resulted in a delayed increase in CEA levels and 10-fold lower plasma levels of CEA. At first glance, it might simply indicate that MV-NIS + MV-CEA–treated mice had minimal or very low tumor burden. Tumor imaging using Tc-99m sodium pertechnetate and actual tumor weights at necropsy indicated otherwise. Peritoneal tumor weights in the MV-NIS + MV-CEA group at the end of the therapy were 2.5 times lower than the MV-CEA group. From these data, we postulate that after i.p. delivery of both viruses, a significant number of tumor cells were infected by both viruses. However, due to the faster replication of MV-NIS virus, the percentage of MV-NIS: MV-CEA–infected cells was skewed toward having more MV-NIS-infected cells, resulting in a delay in increase as well as lower levels of CEA. Indeed, analysis of plasma CEA levels at necropsy confirmed that there was ~80% less CEA per gram of residual tumor in mice that received a combination of MV-NIS and MV-CEA compared with mice that received MV-CEA alone. This competition between the viruses for substrate was manifested in a number of mice that received the combination therapy having very low or undetectable plasma CEA levels.

From this study, we found that gamma camera scans, especially in the orthotopic model of i.p. ovarian disease, are more difficult to interpret and less sensitive than monitoring

---

via plasma CEA. The i.p. tumors were found localized mainly in the greater omentum and there was substantial radionucleide uptake due to normal NIS expression in the stomach and radionucleide accumulation in the bladder. The gamma camera images of i.p. SKOV3 ip.1 tumors were identifiable but required necropsy to confirm presence of these peritoneal tumors. On the other hand, harvest of blood for CEA measurements is easy and clinical laboratories in hospitals have very sensitive assay set up for analysis of plasma CEA levels. One disadvantage of soluble CEA as a marker peptide for noninvasive monitoring is in malignancies, such as colorectal cancer, where CEA is a tumor marker to track disease progression. In those malignancies, MV-bhCG virus might be used instead.

Noninvasive monitoring using soluble CEA revealed a state of equilibrium between viral replication and tumor growth. Indeed, mathematical modeling by Wodarz (33) of tumor therapy with a replicating virus predicted that a state of equilibrium would eventually be reached between growth of viable tumor cells, death of virally infected tumor cells, and viral replication. The CEA profiles in the two groups of measles-treated mice clearly showed this state of equilibrium at the later time points after therapy. This equilibrium is unlikely to occur in an immunocompetent individual as virally infected cells would be cleared by immune cells. We can also potentially affect this equilibrium by a timely application of 131I radioiodine to the MV-NIS-infected tumors for a combination of radiotheroay (12).

In conclusion, we showed, for the first time, that dual therapy with MV-CEA and MV-NIS in an i.p. model of ovarian cancer allowed noninvasive monitoring of virotherapy via soluble marker peptide CEA and gamma camera imaging via NIS. Inclusion of MV-NIS also significantly enhanced the therapeutic outcome of MV-CEA therapy. CEA monitoring is convenient and regular sampling is more feasible and much less expensive than a gamma camera scan. We estimate that 40 CEA tests can be done for the price of a gamma camera image. In addition, sampling can be easily done via a simple blood draw and does not require a hospital visit. Dual therapy with MV-CEA and MV-NIS is thus an attractive option for therapy of ovarian cancer and warrants testing in a Phase I clinical trial.

Acknowledgments

We thank Royce Ruter (Department of Nuclear Medicine) for his expert help in performing the gamma camera imaging, Suzanne Greiner and Mary Harvey (Molecular Medicine Toxicology Laboratory) for their help in vivo experiments, and the Molecular Medicine Program Viral Vector Production Laboratory (Guy Griesmann, Kirsten Langfield, Julie Sauer, Sharon Stephan, Henry Walker, and Troy Wegman) for supply of the MV-NIS virus.

References

Dual Therapy of Ovarian Cancer Using Measles Viruses Expressing Carcinoembryonic Antigen and Sodium Iodide Symporter

Kosei Hasegawa, Linh Pham, Michael K. O'Connor, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/12/6/1868

Cited articles  This article cites 33 articles, 13 of which you can access for free at: http://clincancerres.aacrjournals.org/content/12/6/1868.full.html#ref-list-1

Citing articles  This article has been cited by 7 HighWire-hosted articles. Access the articles at: /content/12/6/1868.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.