Intraperitoneal Therapy of Ovarian Cancer Using an Engineered Measles Virus


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ABSTRACT

The use of replicating viruses for cancer therapy (virotherapy) holds much promise. We reported previously that the live attenuated Edmonston B vaccine strain of measles virus (MV-Edm) had antineoplastic efficacy against hematological malignancies. In this study, we demonstrate that a recombinant MV-Edm, genetically engineered to express an inert soluble marker peptide (MV-hCEA), is potent against human epithelial ovarian cancer cells in vitro and in vivo. The virus was selectively oncolytic for ovarian tumor cells but caused minimal cytopathic damage on non-transformed ovarian surface epithelium and mesothelium. In contrast to nontransformed cells, the ovarian tumor cells expressed high levels of the measles virus receptor CD46. When injected directly into large established s.c. SKOV3ip.1 human epithelial ovarian xenografts in athymic mice, the virus induced complete regression of 80% of the tumors. i.p. administration of virus enhanced the median survival of mice with advanced i.p. SKOV3ip.1 tumors by >50 days. In addition, we could easily follow the kinetic profile of viral gene expression in the treated mice by determining serum levels of the virally encoded marker peptide (soluble human carcinoembryonic antigen). Trackable recombinant measles viruses warrant further investigation for therapy of ovarian cancer.

INTRODUCTION

Epithelial ovarian cancer kills more women than any other gynecological malignancy in the United States. It was estimated that, in 2001, there were 23,400 new cases and 14,000 deaths from ovarian cancer in the United States (1). The disease commonly presents at an advanced stage (I/II; Ref. 2). Early intervention for advanced stage ovarian cancer is debulking surgery followed by chemotherapy with carboplatin/paclitaxel (3). The clinical response rate is 60–70%, but most patients ultimately relapse and succumb to recurrent chemoresistant disease (2). Thus, there is an urgent need for novel therapeutics for the treatment of advanced stage or recurrent ovarian cancer (4–7). Because the disease generally remains confined to the peritoneal cavity, it is particularly amenable to i.p. administration of biologics. The peritoneal cavity serves as a defined compartment to allow maximal interaction/contact between the cancer cells and therapeutic agent. The prospect of encountering neutralizing antibodies is circumvented, repeat dosing is convenient, and toxicity may be less compared with the systemic route. A number of replicating viruses such as adenovirus ONYX-015, reovirus, and herpes simplex virus have been tested for i.p. therapy of human ovarian tumor xenografts in mice and have shown promising oncolytic activities (8–10).

We have been investigating the use of the vaccine strain of measles virus for tumor therapy. Measles is a negative-strand RNA virus (family Paramyxoviridae) that causes rash, fever, cough, coryza, and/or conjunctivitis in an infected person (11). In 1954, Enders and Peebles isolated measles virus from a patient, David Edmonston, and passaged the virus in cultured human and monkey kidney cells (11). Tissue culture passage resulted in an attenuated strain with no pathogenicity (11). From the original Edmonston B strain, a number of vaccine strains were developed and have reduced the incidence of measles infection worldwide (11). In addition to being an effective vaccine, we reported recently that the live attenuated MV-Edm was potent and selective against two B-cell malignancies, non-Hodgkin’s lymphoma (12), and multiple myeloma (13). Infection of lymphoma or myeloma cells in vitro by MV-Edm led to extensive cell-cell fusion and the formation of multinucleated syncytia that eventually became nonviable. Direct intratumoral or i.v. injection of MV-Edm into mice bearing established xenografts resulted in growth inhibition or regression of the tumors (12, 13). These B-cell tumor models were chosen on the basis of the lymphotropism of measles virus. Lymphoid cells are particularly sensitive to MV-Edm infection because the virus binds to a cellular receptor, signaling lymphocyte activation molecule (CDw150), present on activated B and T cells (14, 15). Through tissue culture passage, attenuated vaccine strains of measles viruses have evolved to also infect and enter cells efficiently via a second receptor, CD46 (16–18). Coincidentally, CD46 is overexpressed in human ovarian cancer (19), making it an attractive target for MV-Edm therapy because the virus should enter these receptor-rich cells efficiently. To facilitate the noninvasive monitoring of viral expression profile in treated patients, we developed a recombinant trackable MV-Edm virus expressing an inert soluble marker peptide, the extra-cellular domain of human carcinoembryonic antigen, MV-hCEA (20). We chose hCEA because it has no documented biological activity, the normal serum concentration of hCEA is <5 ng/ml, and 90% of epithelial ovarian cancers do not express hCEA (21–23). Supernatant hCEA peptide levels in MV-hCEA infected cells were concordant with the level of virus gene expression. By monitoring serum hCEA levels in treated rodents, we could obtain a kinetic profile of viral gene expression in a noninvasive manner (20). The virally encoded marker peptide can therefore facilitate dose finding and dose scheduling studies, making it a valuable tool for clinical development of MV-Edm for cancer therapy.

In the current study, we have evaluated MV-hCEA as a potential novel therapeutic agent for i.p. therapy of ovarian cancer. Expression levels of the measles virus receptor, CD46, in a panel of epithelial ovarian cancer cells and nontransformed cells from the mesothelium, ovary surface epithelium, and dermal fibroblasts, were compared by flow cytometry. To determine whether the virus was selectively oncolytic for ovarian tumor cells, the extent of syncytial formation induced by measles virus infection was compared between ovarian tumor cells and nontransformed cells. Antitumor efficacy of the virus was evaluated in an established s.c. SKOV3ip.1 tumor xenograft model and in an advanced i.p. SKOV3ip.1 tumor model in athymic mice. The kinetic profiles of viral gene expression in the MV-hCEA-treated animals were obtained by monitoring serum hCEA levels.

The abbreviations used are: MV-Edm, Edmonston B strain of measles virus; hCEA, human carcinoembryonic antigen; NHDF, normal human dermal fibroblast; OSE, ovarian surface epithelial; FBS, fetal bovine serum; MOI, multiplicity of infection; pfu, plaque-forming unit(s).

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MATERIALS AND METHODS

Cell Culture. The human epithelial ovarian carcinoma cells, OV167, OV17, OV202, OV266, and OV207, were low-passage primary lines established at the Mayo Clinic (24). The SKOV3ip.1 ovarian tumor cells were a kind gift of Dr. Ellen Vitetta (University of Texas Southwestern Medical Center) and MRC-5, NIH:OVCAR-3 cells were purchased from the American Type Culture Collection (Manassas, VA). The NHDFs and vascular smooth muscle cells were purchased from Clonetics and maintained in medium as suggested by the manufacturer (BioWhittaker, Inc., Walkersville, MD). Primary human ovarian carcinoma cells were established in culture from fresh malignant ascites or from disaggregated solid tumors obtained during surgery at the Mayo Clinic. The isolated tumor cells were purified through Ficoll gradients, and identity of the epithelial ovarian carcinoma cells was confirmed by immunostaining for cytokeratin E1/E3 (25, 26). OSE cells were obtained from women undergoing oophorectomies for reasons unrelated to gynecological malignancy and cultured according to Kruk et al. (26). Mesothelial cells were obtained from peritoneal dialysis fluids of cancer-free patients. All primary cultures were used between passages 2 and 3. Permission to obtain and use specimens was reviewed and approved by the Mayo Clinic Institutional Review Board. All ovarian cell lines and primary tumor cells were maintained in alpha-MEM (Irvine Scientific, Irvine, CA) supplemented with 20% FBS (Life Technologies, Inc., Rockville, MD). OSE cells were maintained in 1:1 MCDB 105/Medium 199 supplemented with 15% FBS (26). The Vero African green monkey kidney cells (ATCC CCL-81) used for production of measles virus were maintained in 5% FBS-DMEM (Life Technologies, Inc.). To determine whether any of the cells produced detectable levels of hCEA (limit of detection, >0.5 ng/ml), 2 × 10^5 cells were plated in 6-well plates and incubated in 1 ml of standard medium for 48 h. hCEA levels in the culture supernatants were quantified by the Mayo Clinic Central Clinical Laboratory using the Bayer Centaur Immunoassay System. None of the cells produced detectable levels of endogenous hCEA.

Viruses and Infection Assays. Recombinant MV-Edm encoding the soluble extracellular domain of human CEA (MV-hCEA) was generated and propagated on Vero cells as described previously (18). The titers of viral stocks were determined by 50% end point dilution assays (TCID50) on Vero cells (27). For virus infection assays, 2 × 10^5 cells were incubated with MV-hCEA at a MOI of 0.01 or 0.1 in 1 ml of standard medium for 48 h at 37°C. At the end of the incubation period, the virus was removed, and the cells were maintained in standard medium. At 48 h after infection, the cells were photographed, harvested, and frozen/thawed twice, and virus titer was determined by 50% end point dilution assays (TCID50) on Vero cells (27).

Fig. 1. Ovarian cancer cells express abundant CD46 (A) compared with NHDFs, normal mesothelial cells, and normal OSE cells (B). Cells were incubated with (black histogram) or without (white histogram) antihuman CD46 monoclonal antibody and analyzed by flow cytometry.

Fig. 2. A schematic representation of recombinant MV-Edm with soluble hCEA cDNA inserted as an additional transcription unit. B, increase in viral titer (pfu/ml; ○) was mirrored by a corresponding increase in hCEA (ng/ml; □) levels in cultures of MV-hCEA infected (MOI 0.01) SKOV3ip.1 and OVCAR3 cells.
determined by TCID50 assay on Vero cells. hCEA levels in the culture supernatants were analyzed using the Bayer Centaur Immunoassay System. For cell viability assays, $10^3$ cells/50 μl were plated into 96-well plates and infected with MV-hCEA at different MOIs. At 6 days after infection, the cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Promega Corp., Madison, WI) that measures the formation of insoluble formazan by mitochondria in viable cells.

Flow Cytometry. Cells were harvested, washed twice in cold 2% BSA-PBS, and incubated with mouse antihuman CD46 antibody (PharMingen, San Diego, CA) for 1 h on ice, washed twice, and then incubated with FITC-conjugated antimouse antibody (Sigma Chemical Co., St. Louis, MO) for 1 h on ice. Samples were washed twice and run on a Becton-Dickinson FACScan Plus cytometer and analyzed using the CellQuest software (Becton-Dickinson, San Jose, CA).

In Vivo Experiments. All procedures involving animals were approved by and performed according to guidelines of the Institutional Animal Care and Use Committee of Mayo Foundation. Female athymic mice (5–6 weeks of age; Taconic Laboratory, Germantown, NY) were maintained in the barrier facilities of Mayo Clinic. Mice were implanted s.c. in the right flank with $10^6$ SKOV3ip.1 cells/100 μl PBS or i.p. with $5 \times 10^6$ SKOV3ip.1 cells/250 μl PBS. When the tumors reached 0.5 cm in diameter (11 days after implantation) in the s.c. model, MV-hCEA was injected directly into the xenografts with a 28-gauge needle. Mice in the therapy group ($n = 10$) received a total of five doses of active MV-hCEA ($10^7$ pfu/100 μl/dose), delivered three times in the first week and twice thereafter. Control mice ($n = 10$) were treated with an equivalent dose of virus inactivated by UV irradiation. For the i.p. model, mice received injections i.p. with MV-hCEA ($10^7$ pfu/250 μl/dose) 10 days after implantation of cells. The mice received a total of 16 doses delivered over a 6-week period (total dose, $1.6 \times 10^8$ pfu). Mice in the control group were given UV-inactivated virus. Animals were routinely bled to obtain serum hCEA levels. Animals were euthanized when tumor diameter reached 1 cm (s.c. model) or if mice lost 10% of body weight or developed ascites (i.p. model).

In Situ Hybridization for MV-Edm Nucleocapsid (N) mRNA. SKOV3ip.1 tumors were harvested and fixed in 10% formalin. Paraffin-embedded tissue sections (5 μm) were deparaffinized and were probed for the presence of MV N-specific mRNA using digoxigenin-labeled nucleocapsid RNA of negative polarity as described previously (13).

Rescue of Virus from Residual Tumors. Tumors were harvested and disaggregated under sterile conditions. The suspension was passed through a 40-μm sieve to obtain single cells. Cells were then counted and plated onto tissue culture plates. Virus titer, hCEA level, and presence of syncytia in these cultures were determined.

Statistical Methods. The statistical significance of difference between the survival of mice treated with MV-hCEA or UV-inactivated virus was compared using the log-rank test in the JMP program. $P \leq 0.05$ indicates that the survival of the groups is significantly different.

RESULTS

Ovarian Cancer Cells Express Abundant CD46 Compared with Normal Cells. CD46 expression levels in a panel of ovarian cancer cell lines, SKOV3ip.1, OVCAR3, OV167, OV17, OV202, OV207, primary tumor cells, nontransformed mesothelial cells, nontransformed OSE cells, and normal dermal fibroblasts were compared by flow cytometry. The tumor cell lines OVCAR3, OV167, OV17, OV207 (data not shown) and SKOV3ip.1, OV202 and primary ovarian cancer cells (Fig. 1A), expressed CD46 more abundantly than the

![Fig. 3. MV-hCEA is selectively oncolytic on tumor cells. MV-hCEA infection caused formation of large multinucleated syncytia in ovarian tumor cells (SKOV3ip.1, OV202, and OV207) but has minimal cytopathic effects on nontransformed OSE and mesothelium. Cells were infected at MOI 0.01 and photographed 48 h later.](https://example.com/fig3)

![Fig. 4. Viability of MV-hCEA-infected cells as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Tumor cells were selectively susceptible to MV-hCEA and were killed at lower viral doses compared with normal untransformed cells [NHDF, mesothelial, MRC-5, and vascular smooth muscle cells (VSMC)] at 6 days after infection.](https://example.com/fig4)
MV-hCEA Has Potent Antineoplastic Activity Against s.c. and i.p. SKOV3ip.1 Tumors in Athymic Mice. To investigate the potential of MV-hCEA for therapy of ovarian cancer, we first tested the virus in a s.c. human xenograft model. Human SKOV3ip.1 ovarian tumor cells (5 × 10^6/100 μl of PBS) were implanted in the right flank of athymic mice. Eleven days later when the tumor diameters approximated 0.5 cm, each tumor was injected with a total of five doses of MV-hCEA (10^7 pfu/dose) or an equivalent dose of UV-inactivated virus over 2 weeks. The survival curves of mice in both groups are shown in Fig. 5A. The median survival of mice treated with the active virus was enhanced by >105% compared with the control mice treated with UV-inactivated virus. All mice in the control group were euthanized because of excessive tumor burden by day 41 after implantation of the cells. At that time, 9 of 10 MV-hCEA-treated mice were still alive, and only 1 of the 9 mice had a palpable tumor. Tumors in the remaining 8 mice had regressed completely.

In an orthotopic model of ovarian cancer, 5 × 10^6 SKOV3ip.1 tumor cells were injected into the peritoneal cavities of athymic mice. Ten days later when the i.p. tumors had become well established, mice were randomized to treatment with 10^6/100 μl of UV-inactivated virus or MV-hCEA (10^7 pfu) i.p. Mice that developed ascites or lost ≥20% of body weight were euthanized. The survival curves between the experimental groups are statistically different (P = 0.006). The survival curves of mice in both groups are shown in Fig. 5B. The median survival of mice in the control group was approximately 25 days compared with 30 days in the control group treated with UV-inactivated virus. Eight of 10 mice in the control group had intraperitoneal tumors, whereas none of the 10 mice in the MV-hCEA-treated group had detectable tumors at the end of the experiment. In contrast, all 10 mice in the control group had developed ascites by day 48 after implantation of cells attributable to ascites formation or weight loss as a result of intestinal obstruction by tumor nodules.

Serum hCEA Profiles Helped Elucidate the Kinetics of Virotherapy in Treated Mice. Mice from the treatment and control groups were bled regularly to determine serum hCEA levels during the course of treatment. Rodents are not susceptible to measles virus infection because murine cells lack the appropriate receptor for virus entry. Thus, serum from mice injected with UV-inactivated virus tested negative for hCEA (data not shown). As expected, serum from all tumor-bearing mice that received the active virus became positive for hCEA (Fig. 6). Fig. 6A shows the hCEA profiles of MV-hCEA-treated mice bearing s.c. tumors. In the 8 mice that responded to therapy with complete tumor regression, the corresponding hCEA levels peaked between 10 and 300 ng/ml and subsequently decreased to baseline levels. In contrast, serum hCEA did not return to baseline levels in the two mice with palpable tumors after MV-hCEA therapy, indicating persistence of virus in these nonresponsive tumors.

Serum hCEA levels in mice with peritoneal tumors rapidly increased after virus injections and remained persistently high except in one mouse (mouse 4) in which the serum hCEA peaked at 23 ng/ml and then subsequently decreased to baseline levels (Fig. 6B). The persistence of high serum hCEA levels was unexpected because the mice looked healthy, were free of ascites, and thus were apparently “cured.” To further investigate the cause of this persistent hCEA secretion, the mice were euthanized and evaluated for persistent i.p. tumors. All
mice had residual tumors weighing between 0.07 and 1.3 g, and as shown in Fig. 7, tumor weights were approximately concordant with the serum hCEA levels at the time of postmortem.

In Situ Hybridization and Virus Rescue Assays Revealed Active Virus Replication in Residual Tumors. To determine whether the residual tumors harvested from the peritoneal cavities of the MV-hCEA-treated mice contained live virus, in situ hybridization for measles virus nucleocapsid (N) mRNA was performed on tumor sections. There were scattered positive foci indicating sites of active virus replication throughout the tumor sections (Fig. 8A). High-power magnification revealed that MV-infected cells in the tumor nodule formed large syncytia that stained positive for MV-N mRNA (Fig. 8B). To determine whether MV-hCEA virus would be rescued from explanted tumors, the tumors were disaggregated and plated in tissue culture plates, either on their own or overlaid on Vero cell monolayers. Live virus was rescued in all tumors except from mouse 4. The rescued viruses continued to express hCEA, induced syncytia formation (Fig. 8C), and replicated to produce infectious virions (Table 1). In mouse 4, there was only one very small residual nodule weighing 0.07 g, and serum hCEA levels were below the limits of detection (Table 1), supporting the finding that this nodule did not contain live replicating MV-hCEA virus.

DISCUSSION

This study demonstrates that a recombinant MV-Edm, genetically modified to secrete an inert hCEA marker peptide, is selectively oncolytic for ovarian tumor cells but causes minimal cytopathic effects on nontransformed cells lining the peritoneal cavity (mesothelium and ovarian surface epithelium). The virus showed potent antineoplastic activity against s.c. and i.p. ovarian cancer xenografts, and its expression profile in treated animals could be conveniently monitored in a noninvasive manner through quantitation of soluble hCEA in the serum of treated mice.

It is important to emphasize that it is the tissue culture-adapted attenuated strain of measles virus, not wild-type measles virus, that is being investigated for antitumor activity. Wild-type measles virus is a serious human pathogen and is responsible for a million deaths worldwide each year (11). Most importantly, the tropism of wild-type MV is restricted to lymphoid cells (via signaling lymphocyte activation molecule entry) and produces cytopathic effects only in these cells (28). In contrast, the attenuated MV strains attach and enter cells efficiently through CD46, a ubiquitous regulator of complement activation universally present on all human cells. Ovarian cancer is therefore a particularly attractive target for MV-Edm therapy because ovarian tumor cells, in contrast to nontransformed cells from mesothelium and ovarian surface epithelium, overexpress the CD46 receptor (19). Thus, MV-hCEA is naturally “targeted” to the tumor cells, producing a markedly enhanced cytopathic effect in the ovarian cancer cells versus the nontransformed cells. A single infected tumor cell expressing the viral hemagglutinin and fusion glycoproteins becomes a focus for cell-to-cell fusion with neighboring cells, forming a multinucleated syncytium that eventually becomes nonviable and dies by apoptosis (29). The biochemical mechanisms underlying the remarkable selective oncolytic activity of MV-Edm for tumor cells have not yet been elucidated. However, we are currently testing the hypothesis that CD46 receptor overexpression is a major contributing factor, promoting the process of virus-mediated cell-to-cell fusion.

Fig. 6. Serum hCEA profiles to elucidate the kinetics of MV-hCEA gene expression in treated mice bearing s.c. (A) or i.p. (B) SKOV3ip.1 tumors. B, mice with peritoneal tumors were euthanized, and residual tumors were harvested. The serum hCEA profile of mouse 4 with the smallest tumor burden is highlighted (dashes). No live virus was rescued from the tumor of this mouse.

Fig. 7. Serum hCEA concentration is concordant with the total weight of residual tumors harvested from the peritoneal cavities of mice treated with MV-hCEA at the end of the experiment. The correlation between serum hCEA and tumor weight is $r^2 = 0.9306$.

growth factor, insulin-like growth factor, and single-chain antibodies, and this could offer an additional level of specificity for MV-Edm infection of tumor cells (33, 34).

Serum hCEA levels were persistently elevated in MV-hCEA-treated mice bearing i.p. ovarian cancer cells, although the mice appeared healthy and were free of ascites. Postmortem done on the animals revealed residual tumors, some with necrotic centers, containing foci of MV-hCEA-infected cells demonstrated by in situ hybridization for MV-nucleocapsid mRNA. These sites of viral RNA synthesis appeared as scattered foci in the tumor sections. The explanted tumor cells still expressed CD46 receptors and were able to fuse when plated in tissue culture plates, indicating the presence of virus. Indeed, replication-competent viruses that expressed hCEA were rescued from these tumor cell isolates. Harrison et al. (35) also recovered high levels of titratable adenoviruses from persistent tumors after oncolytic virotherapy in a lung cancer therapy model and attributed the failure of the virus to totally eradicate the tumor because of limitation in viral spread by tumor matrix in vivo. Clearly, in these tumors, there existed a dynamic equilibrium between formation of new tumor cells and death of infected tumor cells. Viable tumor cells continue to divide and contribute to the tumor mass but also provide a substrate for virus replication. Death of virally infected tumor cells keeps progression of the disease in check. This model of a dynamic equilibrium will explain the apparent “cured” status of the mice with persistently high serum hCEA levels. Using mathematical modeling, Wodarz (36) predicted this state of equilibrium of virus persistence in tumors, ongoing death of infected tumor cells, and growth of uninfected tumor cells. According to this mathematical model, the therapeutic outcome will depend heavily on the degree of cytotoxicity of the virus and replication rate of the virus and that of the tumor cells. Obviously, with an intact immune system, the dynamics of virus persistence, infection, and death of infected tumor cells can be altered by cytotoxic T-cell responses and humoral antibodies. It is very likely that in these immunocompetent individuals, virally infected cells/tumor will be recognized by the immune system and will be cleared. Moreover, additional cytotoxic genes can be inserted into the viral genome to enhance/modulate its cytotoxicity to achieve maximal tumor cell killing at the optimal time (36).

Most ovarian cancer patients will have been vaccinated against measles virus and therefore have anti-measles antibodies that might compromise the therapeutic efficacy of the virus. However, neutralizing antibodies would be a more serious concern for systemic therapy than they will be for i.p. therapy of ovarian cancer. Previous studies have shown that the presence of antibodies to replication-competent viruses does not negate the efficacy of therapy or prevent virus replication in the tumor sites (37–39). Indeed, it has been shown that, despite high levels of circulating antibodies in the serum of mice that received multiple injections of adenovirus, the titer of neutralizing antibodies in the peritoneal cavity was undetectable (40). Also, if neutralizing antibodies are present in ascites, the fluid can be easily

With a high level of CD46 receptors on neighboring noninfected tumor cells, binding and fusion with virus-infected cells expressing the measles envelope glycoproteins might be facilitated, amplifying the spread of the virus and cytopathic effects within the culture. In contrast to the MV receptor, the adenovirus receptor, (CAR) is very weakly expressed in ovarian tumor cells (30), which are therefore naturally resistant to infection by oncolytic adenoviruses. To overcome this limitation, significant progress was made in retargeting adenoviruses for entry into cancer cells by adding targeting moieties, such as TAG-72, RGD, and anti-erbB2 single-chain antibody, on the surfaces of the viruses to enhance entry into the ovarian cancer cells (30–32). We have demonstrated recently that it is possible to retarget entry of MV-Edm via display of targeting ligands such as epidermal

Table 1 Live virus was rescued from residual peritoneal tumors

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<th>Mouse ID</th>
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<th>Final serum [CEA] (ng/ml)</th>
<th>Virus titer (pfu/ml)</th>
<th>[CEA] in media (ng/ml)</th>
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Fig. 8. MV-hCEA was detected in and isolated from residual tumors of mice that received i.p. virotherapy. A, in situ hybridization for measles virus nucleocapsid (N) mRNA in tumor sections revealed scattered sites of virus replication (brown staining). Section was counterstained with hematoxylin. B, higher magnification revealed intense staining for MV-N mRNA around the periphery of a syncytium. C, live virus present in residual tumor induced massive cell-cell fusion in the disaggregated tumor cells. Cells were fixed and stained with crystal violet.
aspirated from the peritoneal cavity before therapy. Thus, i.p. therapy is an attractive route for delivery of the virus to maximize virus/tumor cell interaction and infection.

With respect to safety, reversal of attenuated vaccine strains of MV-Edm leading to reemergence of a pathogenic “wild-type” MV has not been observed despite many years of human exposure with live measles vaccine. However, should a pathogenic variant emerge, it is worth noting that measles is a self-limiting disease, and most people recover completely. In addition, caregivers would be expected to have antibodies against the virus, and this would limit person-to-person transmission of the disease.

In conclusion, we believe that MV-hCEA warrants further investigation for i.p. therapy of ovarian cancer. It is potent and selective for tumor cells, and the profile of viral gene expression can be followed through the marker peptide. i.p. delivery circumvents the potential problem that i.p. therapy of ovarian cancer. It is potent and selective for tumor recovery completely. In addition, caregivers would be expected to have antibodies against the virus, and this would limit person-to-person transmission of the disease.

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