Treatment of Intracerebral Neoplasia and Neoplastic Meningitis with Regional Delivery of Oncolytic Recombinant Poliovirus

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ABSTRACT

Purpose: Spread to the central nervous system (CNS) and the leptomeninges is a frequent complication of systemic cancers that is associated with serious morbidity and high mortality. We have evaluated a novel therapeutic approach against CNS complications of breast cancer based on the human neuropathogen poliovirus (PV).

Experimental Design: Susceptibility to PV infection and ensuing rapid cell lysis is mediated by the cellular receptor of PV, CD155. We evaluated CD155 expression in several human breast tumor tissue specimens and cultured breast cancer cell lines. In addition, we tested an oncolytic PV recombinant for efficacy in xenotransplantation models of neoplastic meningitis and cerebral metastasis secondary to breast cancer.

Results: We observed that breast cancer tissues and cell lines derived thereof express CD155 at levels mediating exquisite sensitivity toward PV-induced oncolysis in the latter. An association with the immunoglobulin superfamily molecule CD155 renders breast cancer a likely target for oncolytic PV recombinants. This assumption was confirmed in xenotransplantation models for neoplastic meningitis or solitary cerebral metastasis, where local virus treatment dramatically improved survival.

Conclusions: Our findings suggest oncolytic PV recombinants as a viable treatment option for CNS complications of breast cancer.

INTRODUCTION

Dissemination of cancer to the brain or leptomeningeal invasion of metastatic or primary central nervous system (CNS) neoplasms is usually a terminal event for cancer patients (1), resulting in a median survival of generally <1 year (2–9). This dismal prognosis reflects the limitations placed on radiotherapy by (a) the significant risk of brain damage and spinal cord radionecrosis, (b) the inability to deliver therapeutic drug levels to the brain and cerebrospinal fluid with systemic administration, and (c) the minimal efficacies of currently available chemotherapeutic agents approved for intracerebral or intrathecal use (2–9). As a result, new, potent, and selective treatments for metastatic brain tumors and neoplastic meningitis (NM) are desperately needed.

Recently, genetically engineered human pathogenic viruses have emerged as a novel class of putative antineoplastic agents with properties useful in the design of treatment strategies targeting primary or metastatic CNS neoplasms (10–12). Whereas a variety of viral agents are under consideration for use in such strategies, oncolytic agents based on the neuropathogen poliovirus [PV (13)] may have intrinsic properties that render them particularly suitable for targeting neoplastic lesions arising in the CNS.

The antineoplastic activity of viral oncolytic agents depends on efficient targeting and entry into cancer cells expressing appropriate receptor proteins on their surface. Susceptibility to PV is mediated by the cellular receptor of PV, the immunoglobulin superfamily molecule CD155 (14). Only CD155 gene products in humans (14) and their simian homologs (15) can serve as PV receptors; CD155 alone is sufficient to confer susceptibility to the virus because mice transgenic for the human CD155 gene develop classic poliomyelitis after PV infection (16, 17).

Most importantly, the human pathogen PV needs to be genetically modified for clinical applications to achieve selective virus replication and cytotoxicity in tumor cells without collateral damage to normal CNS or extraneural tissues. Conditional replication in cancerous cells was achieved by genetic recombination with human rhinovirus type 2 (HRV2). PVs and human rhinoviruses, like all Picornaviridae, depend on the internal ribosomal entry site (IRES) within their 5′-nontranslated region for translation initiation of their uncapped (+)-strand RNA genome (18, 19). We have demonstrated that exchange of its cognate IRES with that of HRV2, yielding the chimera PV1(RIPO) (20) and its derivative, PVS-RIPO, drastically reduces PV propagation in cells of neuronal derivation (20). These neural replication deficits ablate the ability of the virus to cause poliomyelitis in CD155 transgenic mice (20, 21) and in intraspinally inoculated cynomolgus macaques (21).

Remarkably, the HRV2 IRES-mediated ablation of neuropathogenic properties does not affect cytopathogenicity for malignant cell types. Selective activity of the HRV2 IRES in
rangingly growing malignant cells suggests fundamental differences in the control of translation rate in cancerous cells, which uniquely exposes these cells to destruction by PVS-RIPO. The molecular mechanisms regulating IRES activity and its cell type-specific restrictions are poorly understood, due to the complexity of potential regulatory influences including canonical translation factors, noncanonical IRES-binding proteins, IRES structure, and interactions with distal regulatory elements within the viral genome (22). We have recently reported that tumor-specific activity of the HRV2 IRES relies on interaction with structural elements of the viral 3'-nontranslated region (23).

In this study, we report the successful use of the prototype oncolytic PV recombinant PVS-RIPO in athymic rat models of breast cancer cerebral metastasis and NM. Intrathecal or intracerebral administration of PVS-RIPO was found highly effective against human breast cancer xenografts growing in the subarachnoid space or brain parenchyma of athymic rats. Our observations suggest that the oncolytic effects of PVS-RIPO are mediated by ectopic expression of the PV receptor CD155 on breast cancer cells. Our approach conceptually resembles successful strategies targeting cell surface molecules of breast cancer cells with monoclonal antibodies (reviewed in Ref. 24). In addition to efficient targeting and destruction of CD155-expressing cancer cells, PVS-RIPO amplification with the possibility of multiple rounds of replication in tumors provides a strong incentive to further investigate the benefits of this novel approach to cancer treatment.

MATERIALS AND METHODS

Human Cell Lines and Virus. Breast cancer cell lines (MDA-MB-361, MDA-MB-468, MDA-MB-231, and Sk-Br-3), glioma cell line U87, and HEK 293 cells were obtained from American Type Culture Collection (Manassas, VA) and propagated according to standard practices. Xenotransplantations were carried out with breast cancer cell line MCF-7/HER2-18, a HER2 cDNA-transfected MCF-7 subclone propagated as described previously (25).

PVS-RIPO was derived from PV1(ROPO) (20) by substituting the coding region of the type 1 live attenuated Sabin [PV1(S)] vaccine strain. Genomic PV1(S) cDNA [provided by A. Nomoto (University of Tokyo, Tokyo, Japan)] was digested with Avai. The resulting 7.0-kb restriction fragment was ligated with a PCR fragment amplified from PV1(ROPO) (22) with primers 5′-GGGTCCATTAATCGACTCTCCTATAGTAAAACAGCTCTGGGTTTG-3′ and 5′-CCATTCTCCGGCAGTGGAGGC-3′ and PBS vector (NEB, Beverly, Massachusetts) digested with Avai and Sall. PVS-RIPO cDNA was processed for re-derivation of virus as described previously (20). UV inactivation of virus (13), synchronized infections (20), and one-step growth kinetics (20) were performed as described previously.

Antigen Capture/Western Blot Analysis and Immunocytochemistry. Breast cancer cell monolayers were homogenized in lysis buffer (PBS and 0.5% NP40). Breast cancer tissues were Dounce homogenized in lysis buffer and centrifuged to remove undissolved debris. The protein concentrations in all lysates were determined using the Bradford method. ELISA plates were treated overnight with monoclonal anti-CD155 antibody D171 (16) suspended in carbonate buffer [0.1 m NaHCO₃ and 0.03 m Na₂CO₃ (pH 9.5)] and rinsed afterward to remove unbound antibody. Individual wells were filled with lysate at the indicated protein concentration and incubated for 4 h at room temperature. After thorough rinsing, the captured antigen was released with SDS-PAGE loading buffer (23) at 95°C. The recovered material was analyzed by SDS-PAGE and Western blot. Filters were blocked overnight in TBST-B [10 mM Tris (pH 8.0), 150 mM NaCl, 0.05% Tween 20, and 3% dry milk] and treated first with polyclonal anti-CD155 antibody D480 (diluted 1:1000 in TBST-B) and then with biotinylated antirabbit IgG antibody (diluted 1:500 in TBST-B; Vector Laboratories, Burlingame, CA) followed by streptavidin-horseradish peroxidase complex (Roche, Indianapolis, IN) and developed with chemiluminescent ECL substrate (Amersham, Piscataway, NJ). Immunocytochemical analysis of polioviral antigens 2C/2BC was performed as described previously (23).

Serial Passaging of PVS-RIPO and Sequencing of the Viral Genome. PVS-RIPO recovered after passaging in MCF-7/HER2-18 cells in culture or obtained from intracerebral xenografts of treated rats was subjected to sequencing by reverse transcription-PCR. Serial passages of PVS-RIPO in MCF-7/HER2-18 cells were carried out as described previously (26). Two rats were sacrificed 14 days after intratumoral virus administration, and the remnant intracerebral xenograft tissue was dissected. The tissue was Dounce homogenized, and the resulting extract was used to infect MCF-7/HER2-18 cell monolayers to amplify virus for further analyses. Viral genomic RNA was recovered from infected MCF-7/HER2-18 cells and subjected to reverse transcription-PCR analysis as described previously (26).

Athymic Rat Model of NM and Metastatic Brain Tumor. Subarachnoid catheters were implanted into female athymic Big:Nimr™ rats (190–206 g) using previously established procedures (25, 27, 28). Briefly, anesthetized rats received a midline sagittal incision from the inion to the laminar arch of C1 to expose the atlanto-occipital membrane and open the underlying cisterna magna dura mater. A PE-10 catheter (Intramedic, Franklin Lakes, NJ) with a 5-0 wire stylet was inserted into the subarachnoid space to the lumbar spinal cord (8.5 cm) and secured with dental epoxy.

Similarly, intracerebral catheters were implanted into the right caudate nucleus using previously established procedures (25). Briefly, a 25-gauge guide cannula (Plastics One, Roanoke, VA) was placed 1 mm anterior, 3 mm right from the bregma and 3 mm deep from the outer table of the skull into the brain of anesthetized rats. The cannula was attached to the skull using cranioplasty cement and sealed with a dummy cannula, and the incision was closed with skin staples. All rats were allowed to recover for at least 7 days, and only animals showing normal weight and neurological function with no evidence of infection were used in the experiments.

MCF-7/HER2-18 cells were harvested with cell scrapers and suspended in PBS to yield a concentration of 10⁶ cells/ml for intrathecal or 8 × 10⁷ cells/ml for intracerebral inoculation. Isolurane-anesthetized rats received 1.25 × 10⁶ MCF-7/HER2-18 cells/100 μl via a Hamilton injector (Hamilton, Reno, NV) intrathecally or 8 × 10⁷ MCF-7/HER2-18 cells/10 μl via a 25-gauge blunt needle 7 mm deep to the outer table of the skull through the guide cannula. Before virus treatment, three ran-
domly selected rats harboring intrathecal or intracerebral xenografts each were sacrificed and examined histologically to confirm tumor presence.

Toxicity and Efficacy Studies. For intrathecal and intracerebral toxicity studies, athymic rats received injection with PVS-RIPO at $10^8$ plaque-forming units (pfu)/60 µl or $10^7$ pfu/10 µl, respectively. Weight, neurological status, survival, and histology of the neaxis and internal organs were compared with a PBS-treated control group.

For intrathecal efficacy studies, rats in the treatment groups received injection with PVS-RIPO at $10^7$ pfu or $10^6$ pfu/40 µl, and rats in the control groups received injection with $10^7$ pfu of UV-inactivated PVS-RIPO. For intracerebral efficacy studies, rats in the treatment groups received injection with PVS-RIPO at $10^5$ pfu/10 µl or $10^4$ pfu/20 µl, and rats in the control groups were injected with $10^7$ pfu of UV-inactivated PVS-RIPO. Weight, neurological status, survival, and histopathology of the neaxis were evaluated in all animals. Neurological evaluation included testing of the stepping and placing reflex and the ability to climb a 60-degree incline ramp. Six representative CNS cross-sections, including forebrain at the level of the lateral ventricles, hindbrain at the level of the occipital lobe, and four equidistant spinal cord/cauda equina sections, were evaluated histologically for hemorrhage, necrosis, edema, demyelination, and arachnoid fibrosis.

Statistical Analysis. Survival estimates and median survival were determined according to Kaplan and Meier (29). Survival data were compared by using the non-parametric log rank test.

RESULTS

Association of CD155 with Breast Cancer Mediates Susceptibility to PVS-RIPO. We used a combined antigen capture/SDS-PAGE-Western blot assay (see “Materials and Methods”) to evaluate the expression of the PV receptor CD155 in a panel of five breast cancer cell lines and normal human breast ductal epithelium (Fig. 1A) and in six breast cancer tissue specimens derived from primary tumor (Fig. 1B). Expression of CD155 could barely be detected in normal human breast epithelium (S2N) with the very sensitive assay used (Fig. 1A; see “Materials and Methods”). CD155 levels in all breast cancer cell lines significantly exceeded the very scant expression in normal breast explant cultures, but they varied considerably among individual cell lines (Fig. 1A). Correspondingly, we observed partial resistance to PVS-RIPO cytolysis in MDA-MB-361, the cell line with the lowest expression of CD155 in the panel (Fig. 2). Infection led to the demise of approximately 70% of these cells by 8–12 h postinfection, but that proportion did not increase, even 24 h postinfection, suggesting resistance to PVS-RIPO infection in subpopulations of this culture (Fig. 2). In contrast, MDA-MB-231 cultures with highly elevated CD155 levels were destroyed completely by PVS-RIPO after 12 h (Fig. 2). The remaining two cell lines were efficiently lysed by PVS-RIPO treatment (data not shown), in accordance with CD155 expression levels in excess of MCF-7/HER2-18.

Variable CD155 expression among established breast cancer cell lines suggests a similar scenario for actual tumor tissues. This assumption was confirmed by analyses of tumor biopsy material, which revealed levels of CD155 expression commensurate with susceptibility to the oncolytic PVS-RIPO in most of the tumors tested (Fig. 1B). Interestingly, in the panel of six tumors analyzed, a range of CD155 expression levels similar to that found among breast cancer cell lines was observed (Fig. 1B). Although direct comparison with cultured cells may be inaccurate due to the complex composition of tumor tissue, scarce CD155 expression in tumors 5 and 6 at levels corresponding to MDA-MB-361 cells may preclude susceptibility to the oncolytic effects of PVS-RIPO. High levels of CD155 expression corresponding to fully susceptible cell lines in most specimens (Fig. 1B) suggest breast cancer cells to be excellent targets for PV-based biotherapeutic agents. The panel of tissue specimens analyzed consisted of primary tumors, and no additional phenotypic or genotypic markers were available. We are currently evaluating a correlation of CD155 expression with additional phenotypic and genotypic markers in a larger set of tumors.
The Dynamics of PVS-RIPO-Mediated Killing of MCF-7/HER2-18. To evaluate the oncolytic capacity of PVS-RIPO in breast cancer cells expressing CD155, we chose MCF-7/HER2-18 with CD155 levels exceeding normal breast epithelium 25-fold but ~2–10-fold lower than MDA-MB-231 and Sk-Br-3 (Fig. 1A). The main motivation for our choice was the fact that MCF-7 is the only line known to form established xenografts after intrathecal administration into athymic rodents (25). Analyses of one-step growth kinetics revealed very efficient propagation of PVS-RIPO in MCF-7/HER2-18 cells in step with replication in highly susceptible U87 malignant glioma cells (13) and HeLa R19 cells used for the propagation of PVs in the laboratory (Ref. 20; Fig. 3A). Efficient particle propagation corresponded to robust viral gene expression in infected cells (Fig. 3B). These findings demonstrate that the cytolytic effect of PVS-RIPO mediated by CD155 coincides with intracellular virus gene expression and particle propagation. Ultimately, the expression of cytopathic viral gene products and rampant particle propagation lead to lytic destruction of tumor cells (see Fig. 2B).

Efficacy of PVS-RIPO against NM in a Human Breast Cancer Xenograft. To test the oncolytic activity of PVS-RIPO against MCF-7/HER2-18-induced NM in vivo, we established intrathecal xenografts in athymic rats as outlined in “Materials and Methods.” Leptomeningeal tumor was evident microscopically 3 days after intrathecal tumor challenge with \(1.25 \times 10^7\) MCF-7/HER2-18 cells and resulted in tumor growth in all rats challenged. All untreated rats died from leptomeningeal tumors (Fig. 4). We evaluated the toxicity of our approach in xenografted rats treated with PVS-RIPO versus PBS as well as efficacy in animals harboring xenografts treated with PVS-RIPO versus UV-inactivated virus.

Because rats do not express CD155 and, hence, are resistant to PV infection, the occurrence of virus-induced toxicity in these animals on infection is unlikely. This expectation was confirmed by the dedicated leptomeningeal toxicity study. Groups of six athymic rats without xenografts receiving intrathecal delivery of either PVS-RIPO \((10^9\text{ pfu in } 60\text{ ml of PBS})\) or PBS alone experienced neither weight loss of >10% nor neurological deficits. Histological examination of the neuroaxis revealed mild peri-catheter demyelination of spinal cord in four rats in the PBS-treated group and two rats in the PVS-RIPO-treated group. These asymptomatic lesions, which are likely due to catheter placement, are commonly seen in studies using this tumor model (25, 27, 28). No evidence of hemorrhage, necrosis, edema, or arachnoid fibrosis was identified in the brain or spinal cord of PVS-RIPO-treated rats, and histological examination of internal organs including heart, lung, liver, spleen, and kidney of each rat showed no abnormalities.

The therapeutic efficacy of PVS-RIPO against MCF-7/HER2-18-induced NM was investigated in groups of 10 rats treated with two different doses administered intrathecally and

Fig. 2 Susceptibility to PVS-RIPO corresponds to relative expression levels of CD155 (compare with Fig. 1A). Cells were infected with PVS-RIPO at a multiplicity of infection of 10 and monitored for 24 h. The arrowheads indicate surviving cells that resist infection. MDA-MB-231 cells were completely lysed by 12 h postinfection, whereas a very minor proportion of MCF-7/HER2-18 cells and a substantial subfraction of MDA-MB-361 cells resisted destruction even 24 h postinfection.

Fig. 3 PVS-RIPO propagation and gene expression in breast cancer cells. A, one-step growth kinetics of PVS-RIPO indicate efficient viral replication in MCF-7/HER2-18 (■), HeLa (○), and U87 (□) cells. B, PVS-RIPO-infected MCF-7/HER2-18 cells stain strongly for poliovirus 2C/2BC antigens.
compared with a group of 10 rats treated with UV-inactivated PVS-RIPO. Treatment with PVS-RIPO 3 days after inoculation of 1.25 × 10^7 MCF-7/HER2-18 cells increased median survival by 130% from 23 days in the UV-inactivated virus-treated group to 53 days in the group treated with 1 × 10^7 pfu of PVS-RIPO (P < 0.0001; Fig. 4). In the group receiving a higher dose of 1 × 10^9 pfu of PVS-RIPO, the median survival was increased to >53 days with a statistically significant survival benefit (P < 0.0001; Fig. 4). There was no statistically significant dose-response relationship (P = 0.0596). Six animals treated with the high dose of PVS-RIPO survived at the end of the experiment and were followed for 173 days after tumor cell inoculation. Of these, one animal harbored residual tumor detected at necropsy.

**Efficacy of Intratumoral PVS-RIPO Injection against Intracerebral MCF-7/HER2-18 Human Breast Cancer Xenografts.** As with NM xenografted animals, intracerebral tumor was also consistently evident microscopically 3 days after intracerebral challenge with the MCF-7/HER2-18 cell line. Furthermore, intracerebral tumor challenges with 8 × 10^5 MCF-7/HER2-18 cells consistently resulted in tumor growth in all rats challenged, and all untreated rats died from intracerebral tumors.

To evaluate toxicity associated with virus treatment, intracerebral administration of PVS-RIPO at a dose of 10^7 pfu/10 μl was compared with treatment with PBS alone in groups of eight and five rats, respectively. At this dose, neither weight loss of >10% nor neurological deficits were observed. Histological examination of brain revealed hemorrhage around the injection site in two rats, possibly caused by the virus injection procedure. There was no evidence of edema, demyelination, or arachnoid fibrosis in the brains of PVS-RIPO-treated rats.

The therapeutic efficacy of intracerebral PVS-RIPO against intracerebral tumor of MCF-7/HER2-18 human breast cancer cells was investigated at two different doses. Treatment of intracerebral MCF-7/HER2-18 human breast cancer cells with 10^7 pfu PVS-RIPO/10 μl 3 days after inoculation of 8 × 10^5 cells increased median survival to 51 days from 23 days in the UV-inactivated PVS-RIPO-treated groups (Fig. 5). These values represent a 122% increase in median survival (P < 0.0001; Fig. 5). In a group receiving a higher dose of 10^9 pfu PVS-RIPO/20 μl, the median survival was increased to 58 days (152%), confirming a statistically significant survival benefit (P < 0.0001) but no statistically significant dose-response relationship. Four rats that received the high-dose virus treatment and two animals treated with low-dose virus survived at the end of the experiment and were followed for 159 days after tumor cell inoculation. Necropsy revealed remaining tumor in one of the animals that had received the low-dose treatment.

**Stability of the Non-Neuropathogenic Phenotype of PVS-RIPO Replicating in Breast Cancer Cells.** To exclude the possibility of PVS-RIPO genetically adapting to neurovirulence on replication in susceptible breast cancer cells, we analyzed virus after 10 serial passages in MCF-7/HER2-18 cells in culture and virus isolated from MCF-7/HER2-18 xenografts of PVS-RIPO-treated rats. Virus recovered after 10 serial passages in MCF-7/HER2-18 cells was subjected to one-step growth curve analysis in MCF-7/HER2-18 and nonpermissive HEK 293 cells (Fig. 6). Furthermore, we performed reverse transcription-PCR sequencing of the genomic region spanning the IRES and the coding region for the capsid proteins for virus recovered after serial passage in culture as well as from PVS-RIPO-treated rats (see “Materials and Methods” for details). Neither the virus passaged in cultured MCF-7/HER2-18 cells nor virus associated with intracerebral MCF-7/HER2-18 xenograft tissue in treated rats differed in sequence from the original inoculum. As expected, one-step growth kinetics in

Fig. 4 Intrathecal treatment with PVS-RIPO of human breast cancer neoplastic meningitis in an athymic rat model. Groups of 10 athymic rats harboring intrathecal MCF-7/HER2-18 xenografts (see “Materials and Methods”) were treated with PVS-RIPO administered at a dose of 1 × 10^7 plaque-forming units (pfu)/40 μl (△) or 1 × 10^9 pfu/40 μl (●), 3 days after tumor cell inoculation. Control animals were treated with UV-inactivated PVS-RIPO (○; corresponding to 1 × 10^7 pfu). Survival was plotted over time.

Fig. 5 Regional treatment with PVS-RIPO of breast cancer cerebral metastasis in an athymic rat model. Groups of 10 athymic rats harboring intracerebral MCF-7/HER2-18 xenografts (see “Materials and Methods”) were treated with PVS-RIPO administered at a dose of 1 × 10^7 plaque-forming units (pfu)/10 μl (△) or 1 × 10^9 pfu/20 μl (●), 3 days after tumor cell inoculation. Control animals were treated with UV-inactivated PVS-RIPO (○; corresponding to 1 × 10^7 pfu). Survival was plotted over time.

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Fig. 6 One-step growth kinetics and plaque phenotype of serially passaged PVS-RIPO. A. Growth kinetics of PVS-RIPO in MCF-7/HER2-18 breast cancer cells (■) and HEK 293 cells (□) before (top panel) and after (bottom panel) 10 serial passages in the former. B. Plaque phenotype of PVS-RIPO before (top) and after (bottom) serial passaging.

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We demonstrate here that the PV receptor CD155, a putative immunoglobulin superfamily cell adhesion molecule, is ectopically expressed in breast cancer cells and a significant proportion of breast carcinomas. In contrast, CD155 levels were exceedingly low in organoid cultures of normal human breast epithelium. Physiological CD155 expression occurs in the embryonic ventral neuraxis (35) and may be responsible for PV’s predilection for anterior horn motor neurons (35). As with many immunoglobulin superfamily cell adhesion molecules (36), including the rodent CD155 relative Tage4 (37), ectopic CD155 expression has been reported in a number of malignancies including colorectal (38) and neuroectodermal tumors (13). This is supported by evidence for CD155 regulation by the morphogenic factor sonic hedgehog and its downstream effectors of the gli family (39). Abnormal activation of sonic hedgehog signaling pathways, which are physiologically active during embryogenesis, is a common feature of many carcinomas (40).

Our analyses of the association of CD155 with breast cancer have revealed important limitations of virus-mediated oncolytic therapy. Low expression in several cell lines correlated with resistant subpopulations that survived PVS-RIPO treatment. Accordingly, scant CD155 levels in some tumors indicate that PVS-RIPO may not be a suitable targeting agent for all breast cancers. Evaluation of the relationship of CD155 expression and PV susceptibility in primary explant breast cancer cultures could lend further support to our findings. However, the enormous difficulties in subcultivating primary breast cancer cells and generating culture systems amenable for virological assays (reviewed in Ref. 41) have prevented us from pursuing this. As with other biological therapeutics targeting ectopically expressed cell surface molecules (24), any clinical application of PVS-RIPO against NM or cerebral metastasis secondary to breast cancer would require tumor profiling to determine CD155 expression levels.

Because our expression analyses rely on tissue homogenates, complementary immunohistochemical assays to elucidate the intratumoral distribution of CD155 would be highly desirable. Immunohistochemical detection of CD155 has been notoriously difficult, and we have been consistently unable to demonstrate CD155 expression patterns unambiguously in human tissues, including the spinal cord anterior horn. This difficulty is likely to result from the exceedingly low levels of CD155, even in high CD155-expressing tumor cell lines exquisitely sensitive to PVS-RIPO [e.g., HeLa cells (42) and MDA-MB-231 cells]. Low CD155 expression levels call for the cumbersome combined antigen capture/Western blot procedure, allowing quantitative detection of CD155 in large samples (>25 μg protein) through prior concentration by antigen capture.

NM and intracerebral metastasis represent lethal final common pathways for a spectrum of malignancies arising in the brain or systemically. The inefficiency of currently available treatment options is a reflection of the unique anatomical structure and physiological conditions prevailing in the CNS (43). Regional delivery, such as injection of therapeutic agents into the interstitium of the tumor or subarachnoid space, bypasses the blood-brain barrier and provides high concentrations of the agent at the tumor site while minimizing systemic exposure (43–46).

Although CD155 expression occurs in primary breast can-
cer tissues, we believe our strategy to be most appropriately directed against metastatic CNS disease. Systemic immunity to PV is universal in vaccinated communities and would be expected to interfere with systemically administered oncolytic agents. However, because immunity to PV extends to the CNS only after PV neuroinvasion, regional intrathecal/intracerebral delivery of oncolytic PV recombinants may provide a unique opportunity to target CD155-expressing cancerous cells with circumvention of preexisting immunity.

Although regional administration of PVS-RIPO improved the survival of NM or intracerebral metastasis xenografted rats, treatment efficacy was not concentration dependent. An incomplete response to virus inoculation could be due to the expansion of virus-resistant populations (as observed in virus-infected monolayer cultures; Fig. 2), possibly relating to low or irregular expression of CD155 in MCF-7/HER2-18 cells (Fig. 1A). Alternatively, more efficient means of intrathecal or interstitial PVS-RIPO delivery, e.g., continuous subarachnoid irrigation with artificial cerebrospinal fluid containing PVS-RIPO or convection-enhanced interstitial administration, may improve intrathecal or intratumoral delivery. We are currently evaluating alternative delivery modes experimentally. Our present studies suggest that intrathecal or intracerebral delivery of PVS-RIPO may be an efficacious and safe treatment of CNS metastasis secondary to breast cancer.

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