Novel cancer treatments are needed to minimize deleterious effects and improve outcomes. Targeted lysis of cancerous cells by viruses without lysis of healthy cells (ie, oncolytic virotherapy) is a promising treatment option that may be more effective and have fewer adverse effects than traditional cancer treatments. A key factor in the success of oncolytic virus treatments is the susceptibility of neoplastic cells to viral infection because of mutations in genes required for function of antiviral defense mechanisms. Some of these mutations also alter cell signaling pathways, which confer selective growth advantages on cells. For example, cells of many tumors have upregulated expression of phosphorylated Akt, which contributes to altered gene expression.
Poxviruses have characteristics that make them excellent candidates for use in oncolytic virotherapy. These viruses preferentially infect cells in tissues with permeable vasculature, which is a feature of many aggressive tumors. Additionally, they can be genetically engineered to express antitumor agents without constraints on gene size, which is a limitation of many other viral vectors used for oncolysis. Poxvirus infection elicits strong cell-mediated immune responses, which prevent establishment of latent or recurrent viral infections.

One poxvirus, MYXV, causes a lethal disease called myxomatosis in European rabbits (Oryctolagus cuniculus). Myxoma virus is nonpathogenic in all other vertebrate species investigated, including humans and dogs. Myxoma virus productively infects cells of multiple human tumor cell lines in vitro. Its effectiveness as an oncolytic virus is supported by findings of other studies in which MYXV treatment was determined to be efficacious in mice with xenografts of human glioma and medulloblastoma. In immunocompetent animals, MYXV oncolysis is more efficacious when used in combination with the chemotherapeutic drug rapamycin.

Administration of chemotherapeutic drugs to animals with tumors might not be necessary if effective oncolytic viruses were available. As with all poxvirus-infected cells, MYXV-infected cells express several immunomodulatory proteins, some of which inhibit apoptosis. Although beneficial for viral pathogenesis during natural infections, expression of such genes is likely to hinder MYXV-based oncolysis. Thus, modification or deletion of antiapoptotic virulence factors in viruses may enhance apoptosis of tumor cells infected by those viruses. Indeed, VACV vectors that do not have the antiapoptosis genes SPI-1 and SPI-2 have enhanced oncolytic specificity and efficacy in mice with adenocarcinoma. However, because VACV is a pathogen of humans and mice, such mutant VACVs replicate in nontumorous cells in several organs, including the brain. Unlike VACV, MYXV does not cause disease in any species of animal except rabbits, which makes production and use of a recombinant MYXV with low replication efficiency unnecessary. Therefore, we wanted to determine the oncolytic properties of a recombinant MYXV that lacks expression of serp2, an antiapoptotic virulence factor. This virus causes milder disease in rabbits than wild-type MYXV but replicates at the same rate in rabbit cells in vitro and in vivo. Testing of viruses for oncolytic efficacy is typically performed with mice that have induced or implanted neoplasms. This may not be the best method to evaluate oncolytic viruses for treatment of naturally developing tumors in humans. Investigation of oncolytic efficacy of viruses in dogs may be a superior method because dogs, like humans, naturally develop neoplasms. Furthermore, because dogs live in close contact with humans, neoplasms in dogs and humans that are caused by environmental factors may have similar biological behaviors. The purpose of the study reported here was to determine the oncolytic efficacy of MYXV in cells of established canine tumor cell lines and in primary canine tumor cells obtained from tumor explants and to compare results with those for MYXV::serp2 to determine whether MYXV-mediated oncolysis is enhanced by removal of the serp2 gene.

Materials and Methods

Sample—Cells from the following established cell lines were obtained: rabbit kidney epithelial RK-13, human malignant glioma U-118 MG, canine osteosarcoma Abrams, canine transitional cell carcinoma Billey, canine osteosarcoma D-17, canine hemangiosarcoma Den, and canine hemangiosarcoma Fitz. In addition, primary tumor cells were isolated from naturally developing tumors surgically removed from dogs that were patients at the University of Illinois Veterinary Teaching Hospital. These tumors included 2 mast cell tumors and 1 each of hemangiosarcoma, soft tissue sarcoma, gastrointestinal stromal tumor, hemangioma, perianal adenocarcinoma, perianal adenoma, mixed mammary gland tumor, and renal carcinoma. Cells were also isolated from noncancerous fibrous connective tissue obtained from the cadaver of a dog undergoing necropsy for reasons unrelated to this study. Unfixed portions of each tumor surgically removed from dogs were dissected free of fat and necrotic tissue. These tumor sections were then minced into 1-mm cubes, placed in a 25-cm² cell culture flask, and incubated at room temperature (approx 22°C) for 10 minutes to allow tissue to adhere to bottoms of the flasks. Then, minimal essential cell culture media with Earle salts, 2 mM glutamine, 50 μg/mL of penicillin G/mL, 50 μg of streptomycin/mL, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids (MEM) containing 10% FBS were added and flasks were incubated (37°C, 5% CO₂, and 100% humidity). Cells from noncancerous fibrous connective tissue (ie, fibroblasts) obtained from the cadaver of a dog were isolated in a similar manner. Trypsinization and passage of cultured cells was performed when cells were 95% to 100% confluent or sooner if tissue explants were degrading. Canine primary tumor and fibroblast cell cultures and cells from established cell lines were maintained in MEM with 10% FBS at 37°C, 5% CO₂, and 100% humidity.

Identification of tumor and cell types—Portions of each tumor surgically removed from dogs were fixed in neutral-buffered 10% formalin and submitted to the University of Illinois Veterinary Diagnostic Laboratory for histologic analysis. Tumors were paraffin embedded, sectioned, stained with H&E, and evaluated to identify tumor types. Primary tumor cells isolated from tumors of dogs were cultured in 35-mm-diameter plates until they were 90% to 100% confluent. Cells were trypsinized, collected in MEM with 10% FBS, and pelleted by centrifugation at 900 X g for 5 minutes. Cell pellets were suspended in 1 mL of PBS solution, and 100-μL aliquots of cells were cytocentrifuged at 400 X g for 3 minutes onto charged glass slides. To ensure cultured cells were representative of the types of tumors from which
they were isolated (rather than adjacent nonneoplastic tissue), cells adhering to slides were evaluated after staining with Wright-Giemsa and cytochemical stains.

**Virus isolation**—Isolation and characterization of MYXVAserp2 (previously referred to as MYXVAsERP2::lacZ) was performed as described. Two recombinant MYXVs (MYXV-gfp and MYXV-red) were also isolated; regulation of each fluorescent protein in these recombinant viruses was under the control of a synthetic vaccinia virus early-promoter.

**Determination of CPEs of viruses**—Primary canine tumor cells and fibroblasts were inoculated with viruses. For comparison with results for canine primary tumor cells, RK-13 (fully permissive for virus replication) and U-118 MG (semipermissive for virus replication) cells were inoculated with viruses. Cells were grown to confluence in 35-mm-diameter plates and inoculated with MYXV, MYXV-gfp, MYXV-red, or MYXVAserp2 at an MOI of 0, 0.1, 1, 3, or 10 infectious virus particles/cell. A low MOI of 0.1 infectious virus particles/cell was included to simulate likely in vivo virus particle-to-tumor cell ratios because sufficient numbers of infectious virus particles cannot be delivered homogenously in a solid tumor in vivo to ensure a high MOI. The MYXV-gfp and MYXV-red (recombinant viruses that express fluorescent proteins throughout the life cycle) were used to aid identification of virus-infected cells. Every 24 hours after inoculation, phase and fluorescent microscopic images of cells were acquired with a microscope (magnification, 100%) and software. Fluorescent images of MYXV-gfp–inoculated cells were acquired with a 480/40-nm bandpass excitation filter and an exposure time of 1,500 milliseconds. Fluorescent images of cells inoculated with MYXV-red were acquired with a 560/40-nm bandpass excitation filter and an exposure time of 400 milliseconds. Images were evaluated, and CPE scores of 1+ (≥ 50% of cells adherent to culture plate), 2+ (10% to 50% cells adherent to culture plate), or 3+ (< 10% of cells adherent to culture plate) were determined for each cell culture.

**Akt expression in cells**—Because increased expression of phosphorylated Akt is associated with greater susceptibility to MYXV-induced CPEs in human cancer cells, western immunoblot analysis of lysates of cells of some of the canine primary tumors (hemangiosarcoma and soft tissue sarcoma) and established canine tumor cell lines (hemangiosarcoma Den and transitional cell carcinoma Billey) was performed to determine relative expression of Akt phosphorylated at serine 473; these cells were not inoculated with viruses. For comparison, protein extracted from human glioma U-118 MG cells, a cell line that moderately expresses phosphorylated Akt, was also assayed. Cells were grown in 35-mm-diameter plates to 95% confluency and then collected in cell lysis buffer with protease and phosphatase inhibitor. Total protein concentration was determined with a standard Bradford assay, and 50 µg of protein from each cell lysate was analyzed via SDS-PAGE (10%). The SDS-PAGE–separated proteins were transferred to a nitrocellulose membrane. Membranes were incubated in blocking buffer overnight (approx 16 hours) at 4°C, then incubated with rabbit antiphospho-Akt (serine 473) monoclonal antibody in accordance with the manufacturer's protocol. Membranes were incubated for 1 hour at room temperature with a horse-radish peroxidase–conjugated goat anti-rabbit IgG polyclonal antibody diluted 1:2,000 in blocking buffer. A chemiluminescent western blot detection kit was used to detect antigen-antibody complexes. Membranes were stripped in accordance with the protocol provided by the manufacturer of the detection kit, incubated with rabbit anti-Akt monoclonal antibody following the manufacturers' instructions, and antigen-antibody complexes were detected. Experiments were repeated 3 times. Immunoblots were scanned and analyzed with photo editing software. Images were inverted (ie, grayscale values were reversed), and integrated density measurements were determined for standardized regions of interest including each protein band. Density values for phosphorylated Akt were normalized relative to density values for total Akt to determine mean percentage of phosphorylated Akt in each cell sample.

As a measure of virus maturation, expression of an MYXV protein (M130R) that is expressed during late stages of viral infection of cells was determined. Cells were collected 24 hours after inoculation with MYXV (MOI ≥ 5 infectious virus particles/cell) and processed as described for determination of Akt expression, except that 25 µg of protein from each sample was separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with rabbit polyclonal anti-M130R primary antibodies.

**Virus growth curves**—Growth curve experiments were performed to determine whether MYXV could replicate in canine tumor cells and produce infectious viruses. To determine 1-step virus growth curves, cells of established canine tumor cell lines (hemangiosarcoma Den and transitional cell carcinoma Billey) and primary canine tumor (hemangiosarcoma), primary canine fibroblast, and RK-13 cells were grown to confluence in 35-mm-diameter plates, inoculated with MYXV (MOI ≥ 5 infectious virus particles/cell), and incubated at 37°C in 5% CO2 for 1 hour. Virus inoculum was removed, and cells were washed with PBS solution 3 times. Then, 1.5 mL of MEM with 10% FBS was added to each plate. Cells were harvested with a sterile handheld flexible natural-rubber scraper attached to a glass rod (ie, rubber policeman) 4, 8, 12, 24, and 48 hours later and centrifuged (400 X g for 5 minutes). Cell pellets were resuspended in 1 mL of MEM without serum and stored at −80°C until determination of virus titers. Multistep growth curve experiments were performed to determine whether MYXV could spread from infected cells to adjacent noninfected cells in cultures. Multistep growth curve experiments were performed in a manner similar to that used for determination of 1-step growth curves, except growth curves were not determined for canine fibroblasts but were determined for canine primary soft tissue sarcoma cells, an MOI of 0.1 infectious virus particles/cell was used, and cells were harvested 0 (ie, 1 hour after virus inoculation), 12, 24, 48, 72, and 96 hours after MEM with 10% FBS was added to each plate.
To determine virus titers for 1-step and multistep MYXV growth curves, tumor cell culture samples were frozen and thawed 3 times, sonicated for 1 to 3 minutes in a water bath at room temperature, and a series of 10-fold dilutions were prepared. Four hundred microliters of each diluted sample was added to confluent RK-13 cells in 35-mm-diameter plates. Cells were incubated (37°C in 5% CO₂ for 1 hour) with agitation every 15 minutes, and a 1-mL overlay (1:1 mixture of 1% high–gelling temperature agarose solution and 2X MEM with 20% FBS; heated to 45°C) was added to the plates. Plates were incubated at 37°C in 5% CO₂ for 5 to 7 days. Plaques formed by MYXV infection of cells (small white foci) were counted. Mean ± SEM virus titers were calculated for results of ≥3 experiments and expressed as the number of plaque-forming units per milliliter.

Transmission electron microscopy—To determine whether MYXVs in all stages of morphogenesis were present in cells, MYXV-inoculated RK-13, hemangiosarcoma Den, transitional cell carcinoma Billey, osteosarcoma Abrams, osteosarcoma D-17, and primary canine hemangiosarcoma and renal carcinoma cells were evaluated via transmission electron microscopy. Twenty-four hours after cultured cells were inoculated with MYXV (MOI, 5 infectious virus particles/cell), media and nonadherent cells were collected. Then, adherent cells were removed by trypsinization, pooled with nonadherent cells, and centrifuged (400 × g for 5 minutes). Supernatants were removed, cell pellets were suspended in Karnovsky fixative, and cell samples were stored at 4°C until imaging. Cell samples were shipped on ice packs (approx 4°C) to the University of Florida Interdisciplinary Center for Biotechnology Research, of which the Diagnostics Laboratory was a part, and cell samples were prepared and imaged with a transmission electron microscope and digital camera.

Cell death and apoptosis assays—Because CPEs in cells infected with viruses may be caused by several factors, including cell death, trypan blue exclusion assays were performed to quantify cell death after inoculation with viruses. Confluent RK-13, hemangiosarcoma Den, transitional cell carcinoma Billey, and primary canine hemangiosarcoma and soft tissue sarcoma tumor cells in 35-mm-diameter plates were mock inoculated or inoculated with MYXV (MOI, 5 infectious virus particles/cell), media and nonadherent cells were collected. Then, adherent cells were removed by trypsinization, pooled with nonadherent cells, and centrifuged (400 × g for 5 minutes). Supernatants were removed, cell pellets were suspended in Karnovsky fixative, and cell samples were stored at 4°C until imaging. Cell samples were shipped on ice packs (approx 4°C) to the University of Florida Interdisciplinary Center for Biotechnology Research, of which the Diagnostics Laboratory was a part, and cell samples were prepared and imaged with a transmission electron microscope and digital camera.

Results

Histologic and cytologic characterization of primary tumor cells—Histopathologic diagnoses determined for tumors surgically removed from dogs were summarized (Table 1). Cell morphology and cytochemical staining results were consistent with tumors from which cells had been isolated, except for cells of one of the mast cell tumors, which unexpectedly lacked staining for mast cell tryptase.
MYXV (MOI, 5 infectious virus particles/cell) were summarized (Table 2). Most cells of established canine tumor cell lines and those obtained from primary tumors had CPEs by 48 hours after inoculation, but few CPEs were observed in primary canine fibroblasts obtained from noncancerous connective tissue.

Extensive CPEs were observed in hemangiosarcoma Den, transitional cell carcinoma Bliley, and primary canine soft tissue sarcoma cells 48 hours after virus inoculation, whereas few CPEs were observed in primary canine hemangiosarcoma cells at that time (Figure 1). Expression of recombinant virus–encoded green (MYXV-gfp) and red (MYXV-red) fluorescent proteins was observed in all canine tumor cell cultures evaluated 48 hours after virus inoculation. In contrast, faint red fluorescence was observed in extremely low numbers of primary canine fibroblasts at 48 hours after inoculation with MYXV-red (MOI, 0.1 infectious virus particles/cell), which indicated that viral infection was inhibited in these noncancerous cells. Growth curve kinetics of MYXV, MYXV-gfp, and MYXV-red in RK-

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NA = Not applicable. ND = Not determined.

Figure 1—Representative photomicrographic images of monolayer cultures of various types of cells (cells of established cell lines rabbit kidney epithelial RK-13, human malignant glioma U-118 MG, canine osteosarcoma Den [HSA-Den], and canine transitional cell carcinoma Bliley [TCC-Bliley]; canine primary hemangiosarcoma [HSA-1] and soft tissue sarcoma [STSA-1] cells obtained from tumors surgically removed from dogs; and canine primary fibroblasts obtained from connective tissue of the cadaver of a dog) 48 hours after inoculation with viruses. A—Phase photomicrographic images of mock-inoculated cells. B—Phase photomicrographic images of MYXV-inoculated cells (MOI, 5 infectious virus particles/cell). Notice CPEs in cells. C—Fluorescent photomicrographic images of cells inoculated with MYXV-gfp (cells with green staining) or MYXV-red (cells with red staining; MOI, 0.1 infectious virus particles/cell). D—Representative phase photomicrographic images of cells inoculated with MYXV-gfp or MYXV-red (MOI, 0.1 infectious virus particles/cell). Bar = 0.1 µm.
13 cells were indistinguishable, which indicated the addition of fluorescent markers did not alter in vitro MYXV replication (data not shown).

Relationship between Akt phosphorylation and CPEs in MYXV-inoculated cells—Slightly greater expression of phosphorylated Akt was detected in hemangiosarcoma Den, transitional cell carcinoma Bliley, and primary soft tissue sarcoma cells than in human glioma U-118 MG cells (Figure 2), although results were not significantly different. These cells had moderate to severe CPEs after inoculation with MYXV (Table 2; Figure 1). In contrast, primary canine hemangiosarcoma cells had the lowest expression of phosphorylated Akt and had few CPEs after inoculation with MYXV. These findings indicated that the level of expression of phosphorylated Akt may be related to susceptibility of canine tumor cells to infection with MYXV.

Production of infectious virus particles in canine tumor cells versus that in RK-13 cells—One-step growth curves (MYXV MOI, ≥ 5 infectious virus particles/cell) indicated logarithmic increases in virus titers during 24 hours after inoculation of RK-13, hemangiosarcoma Den, and primary canine hemangiosarcoma cells (Figure 3). However, virus titers typically decreased after that time. High rates of MYXV replication were not detected in transitional cell carcinoma Bliley cells or primary canine fibroblasts. One-step growth curves were not determined for canine primary soft tissue sarcoma cells because of the large amount of virus particles needed to inoculate cells at an MOI ≥ 5 infectious virus particles/cell.

Multistep growth curves (MYXV MOI, 0.1 infectious virus particles/cell) indicated moderate increases in virus titers after inoculation of cells (Figure 3). Moderate virus production was maintained in RK-13 and canine primary hemangiosarcoma cells through 96 hours after virus inoculation, indicating cell-to-cell spread of viruses in these cell cultures. Although an increase in virus yield was detected for hemangiosarcoma Den, transitional cell carcinoma Bliley, and canine primary soft tissue sarcoma cells, virus titers for these cells decreased after 24 hours. This finding was attributed to either rapid reduction in the number of viable cells or minimal cell-to-cell spread of viruses after initial virus replication.
Virion maturation in MYXV-infected cells—Because the lower yield of infectious viruses from canine tumor cells, compared with that from RK-13 cells, may have been attributable to arrest of the virus life cycle because of aberrant virus morphogenesis, virion maturation in cells was determined. Characteristics of cells of 3 MYXV-inoculated canine tumor cell cultures (hemangiosarcoma Den, transitional cell carcinoma Bliley, and primary hemangiosarcoma) were determined by evaluation of transmission electron micrographic images, and results were compared with those for RK-13 cells (Figure 4). Viral crescents, circular immature virions, immature virions with nucleoids, and intracellular mature virions were detected at 24 hours after virus inoculation in all cell cultures examined by transmission electron microscopy. This finding indicated MYXV replication completed a full cycle of maturation within canine tumor cells and the limited infectivity of lysates of MYXV-inoculated cells was not attributable to aberrant virus morphogenesis. In addition, the late-stage MYXV protein M130R was detected in inoculated RK-13 cells (positive control cells) and canine tumor cells, indicating virus maturation developed in canine tumor cells.

Cell death after MYXVserp2 inoculation versus after MYXV inoculation—To determine whether deletion of the antiapoptotic viral serp2 protein from MYXV would result in a microscopically detectable increase in signs of cell damage, CPE results for MYXVserp2-inoculated cells were compared with those for cells inoculated with MYXV-gfp or MYXV-red. More severe CPEs were observed in RK-13, U-118
MG, hemangiosarcoma Den, transitional cell carcinoma Bliley, and canine primary soft tissue sarcoma cells inoculated with MYXV<sup>Δ</sup>serp2, compared with CPEs in those cells inoculated with MYXV (Figure 5). In contrast, CPEs in canine primary hemangiosarcoma cells inoculated with those 2 viruses were similar. More severe CPEs were detected in osteosarcoma Abrams and canine primary perianal adenosarcoma and perianal adenoma cells inoculated with MYXV<sup>Δ</sup>serp2, compared with CPEs in those cells inoculated with MYXV (data not shown). Because 1-step growth curves for MYXV and MYXV<sup>Δ</sup>serp2 in RK-13 cells were indistinguishable, MYXV<sup>Δ</sup>serp2-enhanced CPEs in these cells were likely not attributable to differences in replication kinetics (data not shown).

Percentage of dead cells gradually increased from 0 to 48 hours after mock or virus inoculation in all cell cultures evaluated (data not shown). Significant differences in percentage of dead cells were not detected when results for mock-inoculated cells were compared with those for cells inoculated with a low virus MOI at any time point (data not shown). The percentage of trypan blue-stained (dead) cells in cultures of 5 types of cells (RK-13, hemangiosarcoma Den, transitional cell carcinoma Bliley, and canine primary hemangiosarcoma and soft tissue sarcoma) at 48 hours after mock, MYXV, or MYXV<sup>Δ</sup>serp2 inoculation (high MOI) were compared (Figure 6). Percentage of dead RK-13, hemangiosarcoma Den, and canine primary hemangiosarcoma cells in cultures inoculated with a high MOI of MYXV was slightly lower than that in mock-inoculated cultures, but differences were not significant. A significantly higher number of dead cells was detected in RK-13, hemangiosarcoma Den, transitional cell carcinoma Bliley, and canine primary hemangiosarcoma cell cultures 48 hours after inoculation with MYXV<sup>Δ</sup>serp2 (high MOI) than in cultures of those cells 48 hours after inoculation with MYXV (high MOI).

Hemangiosarcoma Den, transitional cell carcinoma Bliley, and canine primary hemangiosarcoma and soft tissue sarcoma cell cultures had higher percentages of cells in the early stages of apoptosis (ie, cells with positive results for annexin V staining and negative results for 7-AAD staining) 48 hours after inoculation with MYXV<sup>Δ</sup>serp2 than did mock-inoculated (negative control) cultures for TCC-Bliley and HAS-1 cells. Values are mean ± SEM. *Values that are significantly (P ≤ 0.05) different are indicated by brackets. †Positive control sample values are significantly (P ≤ 0.05) higher than negative control sample values. See Figure 1 for remainder of key.

Figure 6—Results of assays to determine death or apoptosis in monolayer cultures of various types of cells 48 hours after mock inoculation or inoculation with MYXV or MYXV<sup>Δ</sup>serp2. A—Results of trypan blue dye exclusion assay for detection of cell death. An MOI of 10 (MYXV) or 5 (MYXV<sup>Δ</sup>serp2) infectious virus particles/cell was used. Results are mean ± SEM percentage dead cells for ≥3 experiments. B—Representative flow cytometry scatterplots. An MOI of 5 infectious virus particles/cell was used for both viruses. Cells were stained with annexin V and 7-AAD. Cells with positive results for annexin V staining and negative results for 7-AAD staining (ie, apoptotic cells) are shown in the upper left quadrants of the plots. Numbers in quadrants of the plots are the percentage of cells in each of those quadrants. C—Results of flow cytometry analysis indicating percentage of apoptotic cells (ie, cells with positive results for annexin V staining and negative results for 7-AAD staining). An MOI of 5 infectious virus particles/cell was used for each virus. Values are mean ± SEM for results of 2 to 4 experiments. D—Results of DEVD-AMC cleavage assay to detect caspase-3 activity. An MOI of 5 infectious virus particles/cell was used for each virus. Notice that staurosporine-treated (positive control) cultures had higher caspase-3 activity than did mock-inoculated (negative control) cultures for TCC-Bliley and HAS-1 cells. Values are mean ± SEM. *Values that are significantly (P ≤ 0.05) different are indicated by brackets. †Positive control sample values are significantly (P ≤ 0.05) higher than negative control sample values. See Figure 1 for remainder of key.
A much higher percentage of mock-inoculated RK-13 cells had positive results for annexin V staining than was expected on the basis of the finding that CPEs had not been detected in those cells (Figure 5). Therefore, caspase-3 activation assays were performed. Although results were not significantly different, slightly higher rates of DEVD-AMC cleavage by caspase-3 were detected for tumor cells inoculated with MYXVAserp2, compared with that for mock- or MYXV-inoculated cells; this finding suggested that MYXVAserp2 increased apoptosis of canine tumor cells.

**Discussion**

The biological behavior of naturally developing tumors in dogs is more similar to the biological behavior of human neoplasms than is that of induced tumors in mice. Similar to human populations, dog populations may be more likely to develop tumors than is that of induced tumors in mice. Similar to human populations, dog populations may be more similar to the biological behavior of tumors in dogs is more similar to the biological behavior of human neoplasms than is that of induced tumors in mice. In those studies, administration of 1 IV dose of an attenuated VACV (GLV-1h68) to mice resulted in significant reduction in the rate of tumor growth. In addition, the same canine primary soft tissue sarcoma cells used in the present study have been used by a collaborating investigator to evaluate oncolytic efficacy of another strain of VACV for treatment of canine soft tissue sarcoma xenografts in nude mice. Results of that study indicate systemic administration of poxviruses may be efficacious for treatment of dogs with tumors; systemic administration of oncolytic viruses may enable concurrent targeting of primary tumors and metastases. However, immunocompromised mice were used in those studies; such mice do not develop the complex immune responses that develop during viral infection of immunocompetent hosts. Indeed, humoral immune responses to poxvirus infections are strong in susceptible hosts, such as dogs, and humoral immune responses may limit the effectiveness of oncolytic viruses. Despite this possibility, results of clinical trials in which attenuated VACVs were administered to humans with cancer indicate that circulating antibodies to VACV (the smallpox vaccine virus) do not substantially affect treatment outcomes. Similarly, we anticipate that IV administration of modified poxviruses to dogs with cancer would be efficacious, despite clearance of the virus via cell and humoral immune responses.

Results of the present study indicated the poxvirus MYXV, with specific pathogenicity for rabbits, can cause damage and induce expression of viral-encoded proteins in canine tumor cells. In addition, increased expression of phosphorylated Akt seemed to correspond with increased CPEs in canine tumor cells inoculated with MYXV, as has been found for human tumor cells. Furthermore, inoculation of canine tumor cell cultures with MYXVAserp2 increased cell death by apoptosis, compared with results for cells inoculated with MYXV. Additional studies are warranted to determine effectiveness of MYXVAserp2 as an oncolytic agent in vivo. Results of the present study may be relevant to oncolytic virotherapy for humans with cancer.
References


