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Impact of tumor microenvironment on oncolytic viral therapy

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Abstract

Interactions between tumor cells and their microenvironment have been shown to play a very significant role in the initiation, progression, and invasiveness of cancer. These tumor-stromal interactions are capable of altering the delivery and effectiveness of therapeutics into the tumor and are also known to influence future resistance and re-growth after treatment. Here we review recent advances in the understanding of the tumor microenvironment and its response to oncolytic viral therapy. The multifaceted environmental response to viral therapy can influence viral infection, replication, and propagation within the tumor. Recent studies have unveiled the complicated temporal changes in the tumor vasculature post OV treatment, and their impact on tumor biology. Similarly, the secreted extracellular matrix in solid tumors can affect both infection and spread of the therapeutic virus. Together, these complex changes in the tumor microenvironment also modulate the activation of the innate antiviral host immune response, leading to quick and efficient viral clearance. In order to combat these detrimental responses, viruses have been combined with pharmacological adjuvants and “armed” with therapeutic genes in order to suppress the pernicious environmental conditions following therapy. In this review we will discuss the impact of the tumor environment on viral therapy and examine some of the recent literature investigating methods of modulating this environment to enhance oncolysis.

Introduction

The use of viral therapy in the clinic is not a new concept, and has garnered interest for cancer treatment for several decades. In theory, an oncolytic virus (OV) can successfully destroy neoplastic cells while sparing untransformed cells. These viruses have undergone genetic modifications permitting them to infect and/or replicate exclusively in cancer cells. Over the past decade, hundreds of patients in phase I and II trials have been treated with a diverse assortment of OVs. While China has approved the world’s first oncolytic viral therapy for cancer treatment, the United States and Europe are conducting randomized phase III trials to investigate evidence of significant efficacy.¹ As these first-generation oncolytic viruses continue being tested in the clinics, innovative genetic engineering approaches have permitted the design of several second and third-generation viruses, which demonstrate increased

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virulence in neoplastic tissue without compromising safety in animal models.² OV treatment has also shown promise as an adjuvant to radiation therapy and classic chemotherapeutics.³ While advances in research have uncovered several novel anti-neoplastic agents, recent studies have underscored the impact of tumor microenvironment in survival, proliferation, and invasiveness of various cancers.⁴ The tumor microenvironment is constituted of non-transformed host stromal cells such as endothelial cells, fibroblasts, various immune cells, and a complex extra-cellular matrix (ECM) secreted by both the normal and neoplastic cells embedded in it. The significant role played by the tumor microenvironment in viral therapy is just beginning to be understood; this review will focus on changes to the tumor microenvironment subsequent to OV therapy and will discuss recent advances in exploiting these changes in order to craft more effective oncolytic viral treatment strategies.

Angiogenesis and Oncolysis

As tumors grow they require oxygen, metabolites, and waste removal in order to expand beyond a limited size. To achieve this, solid tumors commandeer the host vasculature and initiate the development of tumor vasculature by angiogenesis, the development of new blood vessels from existing endothelial cells, and/or vasculogenesis, a process involving the recruitment of endothelial progenitor cells in order to form new vasculature. Attributes of the tumor microenvironment such as hypoxia, acidosis, inflammation, and oncogene and tumor suppressor mutations disrupt the normal homeostasis maintained between pro and antiangiogenic factors. This allows for increased secretion of pro-angiogenic growth factors, with a concurrent decrease in angiostatic factors, resulting in an “angiogenic switch” in favor of rapid and unrestrained vessel growth.^{5,6}

Blood vessels born from this unregulated process are dilated, tortuous, saccular, and contain numerous arteriovenous shunts. The endothelial cells themselves are atypical in shape, loosely connected, and project into the lumen, causing the tumor vessels to be considerably leaky. Mechanical stress from rapidly proliferating cancer cells also compresses these vessels, contributing to increased resistance to blood flow.⁷ Thus, despite increased blood vessel density, vascular abnormalities promote heterogeneous and inefficient perfusion leading to hypoxia and acidosis throughout the tumor. The poor perfusion and reduced tissue oxygenation contributes to resistance to treatment by radiation, as well as many chemotherapeutics. In this section we will review the literature investigating the impact of OV therapy on the vasculature within the tumor microenvironment and the resulting effects on vascular perfusion.

Recent studies have uncovered both pro and antiangiogenic effects of oncolytic viral therapy on tumoral angiogenesis. Most oncolytic viruses can infect and destroy proliferating tumor endothelial cells, and thus have a direct antiangiogenic effect. Benencia et al. demonstrated the ability of (HSV) 1716, deleted for both copies of the viral $\gamma 34.5$ gene, to infect tumor endothelium and disrupt tumor vasculature both in vitro and in vivo.⁸ Interestingly, this sensitivity towards oncolysis was selective for endothelial cells purified from tumor but not for endothelium derived from normal organs, suggesting targeted destruction of proliferating tumor vasculature. Several other recent studies have corroborated the ability of oncolytic HSV to infect and kill endothelial cells, resulting in a direct antiangiogenic effect.⁹ Dr. Cripe's laboratory at the Cincinnati Children's Hospital in Ohio has also reported a reduction in microvessel density following infection of oncolytic HSV mutants G207 and hrR3 in a malignant peripheral nerve sheath tumor model in mice.¹⁰ More recently, a human telomerase reverse transcriptase promoter-driven oncolytic adenovirus was shown to have antiangiogenic properties mediated by the stimulation of host immune cells to produce endogenous antiangiogenic factors such as IFN- γ .¹¹ While all of these studies demonstrate the ability of OV to infect and replicate in both cancer and endothelial cells surrounding the original injection site, OV infection is also thought to initiate an immune response, ultimately leading to virus

clearance. Thus, tumor cells that are resistant to or have escaped oncolysis regrow following viral clearance in a hypoxic and inflamed tumor microenvironment, both of which are able to alter the angiogenic balance in favor of new blood vessel growth.¹²

While these studies demonstrate the antiangiogenic effects of oncolysis, there also exists contradictory evidence from studies suggesting increased microvessel density in tumors following OV treatment.^{13,14} Recently, oncolytic HSV-1 infection of glioma cells was shown to result in reduction of antiangiogenic proteins TSP-1 and 2. This reduction of angiostatic factors led to a subsequent increase in microvessel density in tumors treated with OV.¹³ Increased microvessel density in tumors following oncolysis was also described by Kurozumi et al., who identified increased expression of pro-angiogenic factors angiopoietin 1 (Ang-1) and CYR61.¹⁴ Both Ang-1 and CYR61 are characterized by their pro-angiogenic signaling effects in the tumor microenvironment.^{15,16} Interestingly, infection of ocular keratitis by wild-type HSV-1 is also known to result in neovascularization of the cornea, and contributes towards the pathogenesis of herpetic stromal keratitis. This has been attributed to increased expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), matrix metalloproteinase 9 (MMP9), and cyclo-oxygenase-2 (cox-2).^{17–19} Collectively, these studies suggest that direct endothelial cell killing by tumor-selective viruses results in a direct antiangiogenic effect. However, after oncolytic destruction and viral clearance, the residual/escaped tumor cells progress in an environment that is conducive for the growth of new blood vessels, leading to increased microvessel density in the recurrent tumor. It would therefore be expected that at early time points following OV therapy a potent antiangiogenic effect would be followed by a phase of blood vessel growth post viral clearance. A recent study by Huszthy et al. compared blood vessel density between treated and untreated tumors in areas with active OV infection and in adjoining uninfected areas. Consistent with the direct antiangiogenic effects of OV, this study revealed a reduction of blood vessels throughout the infected area in treated tumors. Surprisingly, this study also found an increase of angiogenesis in virus-free tumor areas adjacent to infected tissue relative to untreated tumors, indicating a possible shift in homeostasis towards angiogenesis in the tumor microenvironment post oncolysis.²⁰

Apart from the direct endothelial cell killing, OV infection of solid tumors also elicits a potent inflammatory response that can modulate vascular perfusion. Breitbach et al. described a loss of tumor perfusion leading to hypoxic killing of uninfected tumor cells following systemic delivery of vesicular stomatitis virus (VSV) and vaccinia virus in mouse xenograft models.²¹ This loss of perfusion was attributed to virus-induced inflammation, resulting in neutrophil recruitment and clogging of small tumor capillaries. While this vascular shutdown resulted in efficient killing of tumor cells, neutrophil depletion experiments revealed that they also play a significant role in viral clearance and could be limiting for therapy. On the contrary, direct intratumoral delivery of oncolytic HSV-1 in rats bearing intracranial tumors has been shown to result in vascular hyperpermeability and increased vascular leakage.²² Interestingly, like the vascular shutdown observed earlier, the increased vascular leakage has also been correlated with increased leukocyte recruitment and increased inflammatory cytokine release after viral infection. While it is not clear what factors contribute to the very different effects on vascular perfusion, different routes of viral delivery, different tumor sites, and perhaps different types and doses of virus administered in these two studies may contribute to the observed differences.

Anti-angiogenic co-therapies—The impact of OV therapy on tumor vasculature has raised considerable interest with regard to investigating the possible combination of antiangiogenic drug and gene delivery strategies in conjunction with oncolytic viruses. In this section we will summarize how these strategies have been used to combat the aberrant vasculature and perfusion found throughout the tumor, and how these approaches affect the overall efficacy of OV therapy (Table 1 and 2).

Bevacizumab is a humanized anti-VEGF monoclonal antibody, and is the first antiangiogenic agent to be approved by the FDA as an anticancer therapy. Due to its potent VEGF blocking effects, Bevacizumab is able to antagonize vascular permeability and decrease tumor interstitial pressure, leading to a “normalization” of aberrant tumor vasculature.²³ There has, therefore, been considerable interest in evaluating the effect of combining treatment of solid tumors with Bevacizumab and virotherapy. Treatment of mice with Bevacizumab to normalize tumor vasculature prior to intratumoral treatment with E1A-defective oncolytic adenovirus revealed increased viral spread and antitumor efficacy in mice bearing subcutaneous human xenografts.²⁴ This effect is thought to be due to transient vascular normalization and reduced interstitial fluid pressure following anti-VEGF treatment. Contrary to this finding, concomitant treatment of tumor-bearing mice with intraperitoneal delivery of a capsid-modified oncolytic virus and Bevacizumab did not improve survival over single-agent therapy.²⁵ Collectively, these studies underscore the importance of taking into account different tumor models, viruses, and routes of delivery when testing combinational therapeutics prior to translation in human patients. In another study, tumor-bearing mice were treated with vaccinia virus GLV-1h68, Bevacizumab alone, or a single dose of GLV-1h68 followed by twice-weekly treatment with Bevacizumab for five weeks. Concomitant application of a single dose of virotherapy with multiple doses of Bevacizumab yielded better results than either treatment alone.²⁶ Apart from anti-VEGF strategies, co-administration of thrombospondin-derived peptides with oncolytic HSV-1 has been shown to counter the angiogenic response caused by oncolytic HSV-1 treatment, augmenting its overall therapeutic efficacy.¹³ Similarly, treatment of human glioma cells with histone deacetylase (HDAC) inhibitor Trichostatin A (TSA) in conjunction with OV has also been shown to increase the antitumor effects of oncolysis.²⁷ Apart from its direct antitumor effects, antiangiogenic effects of TSA are thought to contribute to the observed in vivo augmentation of efficacy.

Proliferating endothelial cells also depend on integrin ligation and activation for prosurvival signaling. Cilengitide (cRGD) is a cyclic pentapeptide that antagonizes ligation and activation of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$. It is currently being evaluated as an antiangiogenic and anti-neoplastic agent in several ongoing clinical trials for safety and efficacy.²⁸ Kurozumi et al. sought to determine whether reduction in tumor microvessel density achieved by pretreatment with antiangiogenic Cilengitide could reduce the antiviral immune response and augment OV efficacy. Pretreatment of intracranial tumors with cRGD prior to OV infection led to a subsequent decrease in inflammatory cytokines and infiltrating leukocytes, allowing for augmented viral replication.²² In agreement with this, coadministration of Thalidomide has also been shown to increase antitumor effects of OncSyn virotherapy in a syngeneic murine breast cancer model by modulating antitumor immune responses.²⁹

Apart from modulating the tumoral vasculature using antiangiogenic agents in conjunction with oncolytic viral therapy, recent attempts have been made to utilize oncolytic viral vectors to deliver antiangiogenic gene therapy in an attempt to augment antitumor efficacy. Such “antiangiogenic gene armed” OVs target a broad range of factors, including VEGF, matrix metalloproteinases (MMPs), fibroblast growth factors (FGF), antiangiogenic peptides, and interleukins (Table 1). Tumor-selective adenoviruses armed with VEGF targeting genes such as VEGF soluble receptors or SHRNA have been created, and have shown increased efficacy against colorectal renal cell and glioma tumors in mice.^{2,30} Two recent attempts at arming the vaccinia virus with a soluble VEGF receptor 1 and an anti-VEGF single-chain antibody both displayed strong efficacy against human tumors in mice.^{31,26}

Matrix Metallo-Proteases (MMP) are a family of extracellular matrix remodeling enzymes that play a key role in tumor invasion and angiogenesis. Interestingly, while an adenovirus expressing tissue inhibitor of MMP3 (TIMP3) did not reveal increased antitumor efficacy against glioma tumors in mice, expression of TIMP3 in an oncolytic HSV-1-derived virus was

shown to augment therapeutic efficacy against MPNST and neuroblastomas in preclinical models. Similarly, endostatin/angiostatin expression in both adenovirus and HSV-1-derived backbones has been shown to increase potency of the vectors in animal models. Other antiangiogenic strategies exploiting platelet factor 4, dominant-negative fibroblast growth factor and interleukin 8 have also shown increased efficacy in vivo (Table 1). Hardcastle et al. recently created and tested a novel oncolytic HSV-1 vector expressing vasculostatin, a potent brain-specific angiostatic peptide. Treatment of subcutaneous and intracranial glioma-bearing mice revealed potent antitumorigenic effects of this virus compared to control virus.³²

One of the adverse consequences of antiangiogenic therapy is reduced tumor perfusion and resulting hypoxia. While hypoxia is known to modulate tumor cell response to radiation and chemotherapy, its impact on oncolytic viral therapy appears to depend on the type of virus and cell line used. Hypoxia has been shown to reduce viral protein translation following infection, yielding a significant reduction in subsequent viral replication in several group C adenoviruses.^{33,34} Group B adenoviruses maintain their viral protein translation in hypoxic environments, but sustain dysfunction in their lysing ability.³⁵ While reduced oxygen conditions appear to suppress adenovirus replication and oncolysis, enhanced oncolytic HSV-1 replication in hypoxia has been reported.³⁶ In this study, increased expression of cellular growth arrest DNA damage 34 (GADD34) in hypoxia was thought to compensate for the loss of viral γ 34.5 gene.³⁷ Viral γ 34.5 is a neurovirulence gene typically deleted in attenuated oncolytic HSV-1 mutants.³⁸ A combination therapy taking advantage of Cisplatin-induced GADD34 expression was shown to enhance the efficacy of oncolytic HSV-1.³⁹ Apart from GADD34 induction, hypoxia-mediated induction of MEK activity in human breast cancer cells has also been thought to contribute to increased replication of R3616, an attenuated HSV-1-based oncolytic virus.⁴⁰ Similar to HSV-1, vesicular stomatitis virus (VSV) has also been found to possess an inherent ability to replicate in hypoxic tumor cells.⁴¹

Genetic manipulation of OV's has been tested in several preclinical studies to target oncolysis to hypoxic regions within the tumor. HIF-responsive promoters have been used, most notably in adenoviruses, to induce expression of the E1A gene and drive viral replication.⁴² Enhancing the virulence of OV's with hypoxic responsive vectors has shown promising results in numerous cancer cell types and has shown efficacy in both hypoxic and normoxic regions.⁴³

Tumor Extracellular Matrix (ECM)

The extracellular matrix in solid tumors is composed of complex secretions of proteins and proteoglycans produced by both neoplastic and normal stromal cells. These form a complicated network that continuously regulates signaling between tumor and normal stromal cells. Slight changes in the tumor ECM organization can have tremendous impact on cancer cell biology and its response to therapy. Along with increased production of numerous growth factors, cancer cells also increase the secretion of many ECM components. Increased ECM secretion in tumors also results in increased water retention, a significant contributor to increased interstitial fluid pressure. Apart from increased pressure, the interlocked meshwork of secreted proteins presents a physical barrier that interferes with efficient dispersal of therapeutics within the solid tumor.^{44,45} This is bolstered by evidence of only discrete focal viral persistence in tumor xenografts treated with adenovirus in mice.⁴⁶ Viral presence has also been shown to localize only in small discrete areas in clinical tissues harvested from human patients treated with G207.⁴⁷ Efficient oncolysis requires initial entry, followed by viral replication and subsequent release in the tumor environment, in order to infect and lyse surrounding cancer cells. This hinges on the efficient dispersal of virions through the tumor, as inefficient dispersal would result in isolated and sequestered viral replication. These observations indicate that approaches used to modulate the complex extracellular matrix should enhance OV efficacy.

Consistent with this idea, direct intratumoral treatment of subcutaneous tumors in mice with collagenase/dispase and trypsin prior to treatment with beta-galactosidase-expressing recombinant adenovirus resulted in enhanced viral gene transduction within the solid tumor.⁴⁸ Similarly, hyaluronidase-mediated digestion of hyaluronan-rich ECM in human prostate and human melanoma tumors also led to improved intratumoral spread and efficacy of conditionally replication-competent adenoviruses.⁴⁹ The inhibitory effect of collagen on viral spread was demonstrated by Dr. Jain's laboratory using in vivo multi-photon imaging of HSV viral particles with second harmonic generation imaging of fibrillar collagen.⁵⁰ In vivo imaging revealed the presence of HSV virions pooled around extracellular spaces devoid of collagen, indicating that collagen played a significant role in inhibiting virion spread. Additionally, collagenase treatment of melanomas implanted in a dorsal skinfold chamber of SCID mice was found to improve viral dissemination through the tumor. Co-treatment of subcutaneous tumors with oncolytic HSV and collagenase also enhanced virus spread in vivo and translated into better outcomes for tumor-bearing mice.⁵⁰ These results were further corroborated by Dr. Hay's laboratory, where collagen was found to be a significant barrier to viral spread in vitro.⁵¹ Based on these results, Cheng et al. created a non-replicating adenovirus expressing MMP-8, a Zn²⁺ dependant metalloendopeptidase that cleaves types I, II, and III collagen.⁵¹ The resulting AdMMP-8 virus expressed MMP-8 and could efficiently break down collagen. Co-administration of AdMMP8 with a replication-competent virus also enhanced the spread and oncolytic effects, further solidifying the role played by collagen in reducing viral spread.

Based on these observations, gene therapy approaches using proteases and peptides with the ability to modulate synthesis and secretion of ECM have been investigated. Table 3 gives a list of the various oncolytic viruses armed with ECM-modulating genes. Relaxin is a peptide hormone with the ability to decrease synthesis and secretion of interstitial collagens, while increasing the expression of matrix metalloproteinase and procollagenase. Based on these abilities, an oncolytic adenovirus expressing relaxin was engineered and tested for its ability to permit increased viral spread in solid tumors.⁵² Expression of relaxin by the oncolytic adenovirus allowed for enhanced penetration, persistence, and spread compared to the control virus. An adenovirus expressing decorin, a proteoglycan known for numerous ECM remodeling functions, also yielded increased viral penetration and spread.⁵³ Collectively, these studies highlight the need for investigation into the different ECM components found in the tumor and emphasize the importance of exploring additional ways to improve viral spread.

Host Immune responses

Apart from endothelial cells and the secreted ECM, infiltrating host immune cells are another significant component of the tumor microenvironment. While these have the potential to initiate and activate a potent antitumor immune response, they are frequently hijacked by the cancer to produce pro-angiogenic, pro-invasive, and pro-tumorigenic signaling. Upon oncolytic viral infection, changes in secreted cytokines and increased infiltration of immune cells can modulate these responses. While the immune response to OV therapy is generally perceived as an adversary to viral replication, it also has the capacity to re-engage the anti-tumor adaptive immune response, and hence can function as an ally to oncolytic agents. While it is an important aspect of any evaluation of OV therapy, a detailed discussion of the anti-tumor immune response is beyond the scope of this review. In the following section we will briefly discuss the direct contribution of innate immune response to oncolytic viral propagation.

Antiviral cytokines

Similar to viral infection, oncolysis within the tumor elicits a rapid host immune response, resulting in a pervasive antiviral environment. Interferons (IFNs) are a group of antiviral cytokines induced upon pathogen-associated molecular pattern (PAMP) detection by pattern recognition receptors (PRRs). Both groups of PRRs, Toll-like receptors (TLRs) and RIG-I like

receptors (RLRs), are responsible for downstream cascades concluding with the translation of type-I IFN proteins. The IFN response generates a situation where host cells inhibit protein translation, signal for apoptosis, and secrete leukocyte chemoattractants.⁵⁴ Fortunately for OV therapy, most tumor cells lack functional PKR and p53 pathways, and are therefore unable to shut down viral protein translation and induce apoptosis. These distinctions permit attenuated viruses to replicate in neoplastic cells.³⁸ Although innate antiviral responses are somewhat impaired, they can still interfere with viral lysis and spread.

Haralambieva et al. observed significant increases in IFN- α and IFN- β following infection with an oncolytic measles virus.⁵⁵ Activation of the type-I IFN pathway had a powerful influence on restricting production of viral progeny, lysing ability, and spread of the virus throughout the microenvironment. Wild-type measles virus encodes for the P gene, which can antagonize the antiviral effects of IFN induction and/or response. Arming the oncolytic measles virus with the wild-type P gene resulted in enhanced oncolytic potential in vivo. This study highlighted the significant limitations of host innate defense responses with regard to oncolytic viruses. Similarly, oncolytic HSV-1 treatment of glioma has also been shown to result in a significant increase in inflammation and leukocyte infiltration.²² This was attributed to an increase in the expression of IFN- γ and IFN- γ inducible genes CXCL11 and CXCL9, which led to subsequent vascular hyperpermeability in the tumor microenvironment.¹² Increased secretion of antiviral cytokines such as IFN type I and II and TNF- α have direct antiviral effects on infection and replication, and also modulate the recruitment and maturation of invading innate and adaptive immune cells.

Infiltrating Cells

Increased IFN and IFN-inducible chemokines produced during oncolytic viral infection create a pro-inflammatory state that facilitates the influx of natural killer (NK) cells and dendritic cells.⁵⁶ Macrophages and DCs activated by an oncolytic adenovirus can release an array of antiviral and pro-inflammatory cytokines, including IL-12, IL-6, and TNF- α .⁵⁷ These pro-inflammatory cytokines influence recruitment and activation of a wide variety of immune cells including macrophages, NK cells, neutrophils, CTLs, and other antigen-presenting cells, all of which have been implicated in viral clearance.⁵⁸

Monocytes/Macrophages are a diverse group of phagocytic cells that upon activation phagocytose intruding microbes and release a diverse array of cytokines and chemokines, quickly altering the immune milieu. Dr. Chiocca's laboratory at The Ohio State University has extensively studied the infiltration and impact of both resident microglia and infiltrating macrophages following OV therapy in intracranial glioma models.⁵⁹ Depletion of these macrophages and microglia resulted in higher viral titers throughout the tumor, underscoring the importance of these cells in viral clearance. Similarly, a significant accumulation of neutrophils has also been observed post VSV infection, and is thought to be partially responsible for the occlusion of capillaries attenuating blood flow.²¹ While this acute inflammatory response reduced viral titers, transient deletion of peripheral neutrophils prior to infection significantly increased viral progeny throughout the tumor environment. Antigen-presenting dendritic cells have also been shown to mature following OV infection and release cytokines and chemokines that can activate both CTLs and NK cells.⁶⁰ Macrophage and DC-derived cytokines cause a rapid recruitment of NK cells to the infected area. NK cells are non-phagocytic, but destroy viral infected cells through induction of apoptosis. Altomonte et al. recently highlighted the importance of the NK cell response by displaying elevated viral titers and in vivo efficacy following both antibody depletion and VSV vector-mediated inhibition of NK cells.⁶¹

Circumventing the negative effects of the host immune response

Oncolytic viruses face opposition from detection, inactivation and elimination immediately following their entrance into a host. Recently, a number of attempts have been made to suppress the innate antiviral effects of the immune response in order to improve oncolysis throughout the tumor environment. These methods center around the suppression of innate immune cell interactions and signaling in an attempt to conceal the virus from host detection.

Pharmacological—Pharmacological attempts to circumvent the innate immune response have been thoroughly investigated. Cyclophosphamide (CPA) is a DNA alkylating agent that has direct antitumor effects and is also known to reduce neutralizing viral antibodies, allowing for a significant increase in viral progeny and overall efficacy in vivo.^{62,63} CPA treatment prior to oncolytic HSV therapy has been shown to inhibit infiltration of innate phagocytes such as NK cells, microglia and macrophages, allowing for increased viral persistence and improved overall efficacy in a syngeneic rat glioma.⁶⁴ Combination treatment of CPA and the oncolytic HSV rRp450 also showed strong efficacy and safety in an aggressive sarcoma xenograft model.⁶⁵ Similar effects have also been observed with CPA and adenovirus, as well as other immunosuppressants such as rapamycin.^{66,63} CPA has also been shown to suppress Tregs, thus improving antitumor immune responses following OV therapy.⁶⁷ Based on these results, a phase I clinical trial combining the treatment of cyclophosphamide with an oncolytic measles virus is currently underway.⁶⁸ The importance of the innate interferon response has also been shown indirectly through the adjuvant use of histone deacetylase (HDAC) inhibitors. HDAC inhibitors have been shown to have anti-proliferative and antiangiogenic effects, while also preventing IFN-stimulated anti-viral gene expression.⁶⁹ Dampening the interferon response with HDAC inhibitors has shown strong synergy in conjunction with both VSV and HSV-1 derived oncolytic viruses.^{70,71} Collectively, these studies underscore the importance of the antiviral IFN response in tumor cells.

Conclusion

The tumor microenvironment forms a formidable barrier for efficient oncolysis by impeding viral penetration, persistence and spread. While great strides have been made in targeting and arming oncolytic viruses, it is only recently that the importance of the microenvironment in successful virotherapy has been acknowledged. Because of the failures of OV therapy as a single agent, it is likely that future successes will hinge on the adjuvant use of synergistic therapies in an attempt to establish an environment that is permissive to infection, replication, and destruction of tumor cells.

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Biographies

Jeffrey Wojton is a Ph.D student in the Neuroscience Graduate Studies Program at The Ohio State University, where he is working in the laboratory of Dr. Balveen Kaur. He graduated from Marietta College in 2008 with a B.S. in Health Science and is interested in innovative gene therapy techniques for CNS tumors. His research interests focus on the discovery and enhancement of oncolytic viral therapies for glioblastoma.

Dr. Balveen Kaur is an Associate professor in the Department of Neurological Surgery at the Ohio State University, Columbus OH. She majored in Physics at Delhi University and proceeded to obtain an M.S. in biotechnology at Banaras Hindu University. She subsequently carried out her Ph.D. at Emory University, followed by a postdoctoral fellowship in the laboratory of Dr. Erwin Van Meir at Emory University, where she studied the role of angiogenesis in the context of glioma progression. Much of her work focused on the tumor microenvironment and antiangiogenic and antitumorigenic properties of endogenous inhibitors. She joined the faculty of The Ohio State University in 2005 as an Assistant Professor, where her laboratory is currently studying the role of the tumor microenvironment and angiogenesis as limiting factors for glioma virotherapy.

Table 1

List of Oncolytic viruses delivering angiostatic gene therapy

	OV Name	Virus Type	Therapeutic gene	In Vivo Cancer Model	Reference
1	ZD55-VEGF-251	Ad	VEGF 251	Cervical, Colorectal	Xiao T et al. Cell Research 2009; doi: 10.1038/cr.2009.126
2	GLV-1h68	VV	GLAF-1	Pancreatic Carcinoma, Prostate Carcinoma	Frentzen et al. Proc Natl Acad Sci USA 2009;106:12915–12920
3	ZD55-sflt-1	Ad	sFlt-1	Colorectal	Zhang Z et al. Mol Ther 2005;11:553–62
4	Vvdd- VEGFR-1-Ig	VV	sFlt-1	Renal Cell Cancer	Guse K et al. J Virol 2010;84:856–866
5	Ad5/3-9HIF- Δ24-VEGFR-1-Ig	Ad	sF-1-Ig	Kidney	Guse K et al. Gen Ther 2009;16:1009–1020
6	Ad-ΔB7- shVEGF	Ad	shRNA- VEGF	Glioma	Yoo JY et al. Mol Ther 2007;15:295–302
7	AdΔB7-KOX	Ad	F435-KOX	Glioma	Kang YA et al. Mol Ther 2008;16:1033–40
8	AdΔ24TIMP3	Ad	TIMP3	Glioma	Lamfers ML et al. Cancer Res 2005;65:9398–405
9	rQT3	HSV-1	TIMP3	MPNST	Mahler YY et al. Cancer Res 2008;68:1170–9
10	CRAD-Cans	Ad	Canstatin	Pancreatic	Xiao-Ping H et al. Cancer Letters 2009;285:89–98
11	HSV-Endo	HSV-1	Endostatin	Colon Carcinoma	Mullen JT et al. Cancer 2004;101:869–77
12	AE618	HSV-1	Endostatin- Angiostatin fusion Pr.	Lung Cancer	Yang CT et al. Anticancer Res 2005;25:2049–54
13	CNHK500- mE	Ad	Mouse Endostatin	Nasopharyngeal tumor	Su C et al. Mol Cancer Res 2008;6:568
14	CNHK200- mE	Ad	Endostatin	Hepatocellular carcinoma	Li G et al. Int J Cancer 2005;113:640–8
15	RAMBO	HSV-1	Vasculostatin	Glioma	Hardcastle J et al. Mol Ther 2009;Oct 20
16	bG47Δ-PF4	HSV-1	Platelet Factor-4	Glioma, Malignant Peripheral Nerve Sheath Tumors	Liu TC et al. Mol Ther 2006;14:789–97
17	bG47Δ-dnFGFR	HSV-1	dn Fibroblast growth factor	Glioma, Malignant Peripheral Nerve Sheath Tumors	Liu TC et al. Clin Cancer Res 2006;12:6791–9
18	Ad-ΔB7- U6shIL8	Ad	shRNA-IL-8	Liver Cancer, and Non small cell lung cancer, Pulmonary metastasis of breast	Yoo JY et al. Gene Ther 2008;15:635–51
19	M4	Ad	Antisense STAT3 cDNA	Gastric Carcinoma	Han Z et al. Carcinogenesis 2009;30:2014–2022

Abbreviations: VEGF: Vascular Endothelial growth factor; sflt-1: soluble VEGF receptor flt-1; Ad: Adenovirus; sh-VEGF: small hairpin targeting VEGF; TIMP3: Tissue inhibitor of metalloproteinases; HSV-1: Herpes Simplex virus; dnFGFR: dominant negative Fibroblast growth factor receptor; shIL8: small hairpin targeting IL8; GLAF-1: anti-VEGF single-chain antibody; sF-1Ig: soluble VEGFR 1-Ig; RAMBO: rapid antiangiogenic mediated by oncolytic virus.

Table 2

List of studies combining antiangiogenic agents with oncolytic viruses.

	Oncolytic Virus used	Type of virus	Tumor model	Treatment strategy	Efficacy	Reference
Avastin						
1	DJ922-947	E1A deleted Adenovirus	Anaplastic thyroid carcinomas in athymic nude mice	Avastin (i.p.) every 5 th day and OV twice a week for 4 weeks	Co-treatment group enhanced efficacy over single agent alone	Libertini et al. Clin Cancer Res 2008;14:6505–14
2	Ad5/3-Δ24	Ad5 virus with E1 gene with 24 bp deletion, and serotype 3 knob	Peritoneal disseminated Renal Cell cancer in SCID mice	Avastin: i.p. once a week for 5 weeks and OV: i.p. on days 7, 14, and 21 post tumor implant.	No enhancement over OV alone	Guse et al. Mol Cancer Ther 2007;6:2728–32
3	Ad5-Δ24RGD	Ad5 virus with E1 gene with 24 bp deletion, RGD motif in H1 loop of knob protein	Peritoneal disseminated Renal Cell cancer in SCID mice	Avastin: i.p. once a week for 5 weeks and OV: i.p. on days 7, 14, and 21 post tumor implant.	No enhancement over OV alone	Guse et al. Mol Cancer Ther 2007;6:2728–32
4	Ad5.pk7-Δ24	Ad5 virus with E1 gene with 24 bp deletion, and 7 lysine residues at C terminus of fiber	Peritoneal disseminated Renal Cell cancer in SCID mice	Avastin: i.p. once a week for 5 weeks and OV: i.p. on days 7, 14, and 21 post tumor implant.	No enhancement over OV alone	Guse et al. Mol Cancer Ther 2007;6:2728–32
5	GLV-1h68	<i>Renilla</i> luciferase, GFP, and fusion, β-galactosidase, and β-glucuronidase expressing VV	s.c Pancreatic cancer cells in mice	Avastin: twice a week for 5 weeks 13 days post OV treatment; OV: single dose i.v	Co treatment group enhanced efficacy over single agent alone.	Frentzen et al. Proc Natl Acad Sci USA 2009;106:12915–20
Thrombospondin 1 peptide						
5	G207	HSV-1 deleted for ICP34.5 and disrupted for ICP6	s.c glioma in nude mice	TSR peptide: i.p. daily days 1–27. OV: on days 2 and 5.	Co treatment group enhanced efficacy over single agent alone.	Aghi et al. Cancer Res 2007;67:440–4
6	G47Δ	HSV-1 deleted for ICP34.5 and disrupted for ICP6, and deleted for α47.	s.c glioma in nude mice	TSR peptide: i.p. daily days 1–27. OV: on days 2 and 5.	No enhancement over OV alone	Aghi et al. Cancer Res 2007;67:440–4
Trichostatin A						
7	G47Δ	HSV-1 deleted for ICP34.5 and disrupted for ICP6, and deleted for α47.	s.c glioma in nude mice	TSA: i.p. daily for twelve days; OV: i.t on days 1, and 4.	Co treatment group enhanced efficacy over single agent alone.	Liu et al. Mol Ther 2008;16:1041–7
Cilengitide						
8	hrR3	HSV-1 deleted for ICP34.5 and disrupted for ICP6, and deleted for α47.	i.c rat glioma in rats	Cilengitide: i.t on day 3; OV: i.t day 7	Co treatment group enhanced efficacy over single agent alone.	Kurozumi et al. J Natl Cancer Inst. 2007;99:1768–81
Thalidomide						

	Oncolytic Virus used	Type of virus	Tumor model	Treatment strategy	Efficacy	Reference
9		HSV-1 +/- forlCP34.5 and mutant gB and gK.	s.c 4T1 tumors in mice	Thalidomide: orally in chow from day 4; OV: 1x three times	Co treatment group enhanced efficacy over single agent alone.	Israyelyan et al. Cancer Chemother Pharmacol 2009;64:1201–10

Abbreviations; i.v: intra venous; s.c: sub cutaneous; i.p: intra peritoneal; i.c: intra cranial; Ad: Adenovirus; VV: Vaccinia virus

Table 3

OV armed with matrix modulating enzymes

OV NAME	VIRUS TYPE	THERAPEUTIC GENE		IN VIVO MODEL	REF.
Collagen Targeting OV:					
Ad-ΔE1B- DCNG	Ad	Decorin	Modulation of several ECM components	Glioma, Metastatic Melanoma	Choi IK et al. Gen Ther 2009;Nov 17:1–12
Ad-ΔE1B- RLX	Ad	Relaxin	Collagen modulation	Hepatocellular carcinoma, and lung metastasis	Kim JH et al. J Natl Cancer Inst 2006;98:1482–93