The use of engineered viruses to treat tumors is one of the many therapies progressing from the laboratory to the bedside. Even with the most meticulous technique, neural damage is an inherent risk in the surgical management of peripheral nerve tumors, and new avenues of treatment with reduced morbidity are constantly being sought. In this review we present the use of genetically engineered HSVs as oncolytic vectors in the management of peripheral nerve tumors.

We have long known about the ability of viruses to act as oncolytic agents. In 1904 Dock, a professor of medicine at the University of Michigan, reported a case of a 42-year-old woman with myelogenous leukemia whose symptoms and white-cell count improved during a bout of influenza, only to deteriorate again afterwards. However, it was not until 1991 that the first description of a genetically engineered HSV with selective replication in dividing cells and tumor oncolytic ability was reported. The initial model was developed in glioma cell lines, but since then the field has progressed rapidly and now encompasses many tumor models, including meningiomas, mesotheliomas, and tumors of the prostate, thyroid, liver, and peripheral nerves.

In this review we will examine the mechanisms by which oncolytic HSV mediates its antitumor effect and the main strategies that are implemented to increase its efficacy without compromising its safety. In addition, we present the evidence for its safety that has allowed a nervous system pathogen to join the ranks of cancer therapies that have entered clinical trials in humans.

Herpes simplex virus is able to infect a wide array of cell types in various species, and the availability of well-established antiviral therapies to treat it. The efficacy of oncolytic HSV therapy against schwannomas and malignant peripheral nerve sheath tumors has been studied in multiple experimental models both in vitro and in vivo. The virus utilizes cell pathways unique to tumors to enhance its oncolytic efficacy, preferentially and effectively targeting and destroying peripheral nerve tumor cells without harming normal cells. This effect is augmented by transgenes expressing antiangiogenic factors, such as dominant-negative fibroblast growth factor receptor and platelet factor 4, and displays synergy with chemotherapy. Different oncolytic HSV vectors have been tested, including hrR3, G207, and G47. In addition, new animal models have been developed to test the efficacy of oncolytic HSV therapy in peripheral nerve tumors. The safety of oncolytic HSV is well-established and has been tested in nonhuman primates and in human clinical trials.

**Key Words**

- herpes simplex virus
- malignant peripheral nerve sheath tumor
- oncolytic therapy
- peripheral nerve tumor
- schwannoma

Abbreviations used in this paper: dnFGFR = dominant-negative fibroblast growth factor receptor; eIF2α = eukaryotic initiation factor 2α; HSV = herpes simplex virus; MOI = multiplicity of infection; MPNST = malignant peripheral nerve sheath tumors; PF4 = platelet factor 4; pfu = plaque-forming units; PKR = double-stranded RNA-dependent protein kinase.
types in various species. The HSV genome contains 152 kb of DNA and has been sequenced. In addition, the function of its genes has been studied extensively and key neurovirulence genes identified. About 30 kb of DNA is considered nonessential for viral replication in tumor cells and can be replaced.\textsuperscript{8} The nonessential part of the genome provides the opportunity for the insertion of DNA coding for transgenes or the so-called “arming” of the virus to augment its antitumor effect. Furthermore, a key attribute of HSV is its ability to deliver transgenes effectively into the nucleus of cells without integrating into the host DNA, thereby reducing the risk of insertional mutagenesis. Moreover, in the event of adverse reactions, well-established antiviral drugs like acyclovir can be administered.

Oncolytic HSV effects its antitumor activity by selectively replicating in tumor cells but not in normal cells. As part of the life cycle of the virus, cell destruction and viral spread occur, allowing more tumor cells to be infected and the antitumor effect to be propagated. In addition, many novel strategies to enhance the killing of tumor cells have been developed, and some have been studied in peripheral nerve tumor models. A common theme is the use of viral vectors expressing transgenes. One example has been the use of oncolytic HSV expressing interleukin-12,\textsuperscript{2,28} the aim of which is to recruit the immune system to enhance the effect of the viral therapy. Another strategy is to establish synergy with existing chemotherapy and radiotherapy regimens.\textsuperscript{2,14}

Prodrugs have also been used in combination with HSV expressing transgenes for prodrug activation. Tyminski and colleagues\textsuperscript{26} described one example in gliomas, using the prodrugs cyclophosphamide and irinotecan and oncolytic HSV expressing multiple prodrug activation transgenes. Using this combination, the authors demonstrated enhanced oncolysis without affecting viral replication. Oncolytic HSV therapy has also been used to target tumor angiogenesis.\textsuperscript{1,5} With this therapy, the aim is not only to prevent tumor angiogenesis but also to cause regression of the tumor blood supply. This tactic has been employed in peripheral nerve tumor models.

**Oncolyisis of Peripheral Nerve Tumors**

In 2001, the first report of a genetically engineered virus inoculated into a peripheral nerve was reported.\textsuperscript{22} In this study, Mashour et al.\textsuperscript{22} described the use of the selectively replicating HSV G207 for oncolysis in human MPNST-derived ST8814 cells and human neuroblastoma–derived IMR32 cells. The authors also demonstrated this vector’s safety when inoculated into peripheral nerves.

The G207 variant was constructed from HSV wild-type strain F and has a number of mutations engineered for neuronal safety. It has deletions of both copies of $\gamma_{34.5}$ located in the inverted repeat sequences flanking the unique long segment of the viral genome and an inactivating insertion of the \textit{E. coli lacZ} gene in the UL39 locus.\textsuperscript{27} Herpes simplex virus with deletions in both copies of $\gamma_{34.5}$ have markedly reduced neurovirulence\textsuperscript{28} but retain the ability to replicate in tumors. The $\gamma_{34.5}$ gene also prevents the host cell from shutting down protein synthesis in response to HSV infection.\textsuperscript{1} The UL39 gene codes for the large subunit of HSV ribonucleotide reductase, which is required for viral DNA synthesis. The inactivation of UL39 by the insertion of the \textit{E. coli lacZ} gene results in preferential replication of the virus in tumor cells where mammalian ribonucleotide reductase levels are elevated. In addition, \textit{lacZ} functions as a reporter gene that allows monitoring of virus spread.

In safety studies, G207 was injected into the sciatic nerve of mice at $5 \times 10^6$ pfu and compared to wild-type HSV strain F and control.\textsuperscript{21} In mice injected with strain F, widespread signs of myelitis, meningitis, and encephalitis were noted in the central nervous system.\textsuperscript{22} In contrast, in the mice injected with G207, the signs of inflammation were restricted to the ipsilateral sciatic nerve.

The efficacy of this vector was tested both in vitro and in vivo. In vitro, the efficacy of G207 was demonstrated at an MOI of 0.1, killing 80% of ST8814 cells by Day 5 and 90% of IMR32 cells by Day 2.\textsuperscript{22} In vivo evaluation was undertaken with IMR32 cells only because the ST8814 cells were unable to generate detectable tumors. Again, G207 demonstrated efficacy with only a single injection of $10^6$ pfu, leading to decreased neurological deterioration and 30% survival in the treated group compared with 10% in the untreated group.\textsuperscript{22}

In 2006, Messerli and colleagues\textsuperscript{26} described the oncolysis of schwannomas in mice in both transgenic and human xenograft models using G47Δ. A derivative of G207, G47Δ has an additional deletion of the $\alpha_{47}$ gene.\textsuperscript{3,24} The product of $\alpha_{47}$ binds to the transporter associated with antigen presentation and prevents HSV antigen processing in the endoplasmic reticulum and presentation by major histocompatibility complex class I molecules.\textsuperscript{12,25} This deletion enhances immune presentation in human tissue and suppresses the attenuated replication properties of $\gamma_{34.5}$ mutants in all species tested.

The transgenic model described by Messerli et al.\textsuperscript{26} uses immune-competent mice that spontaneously form tumors because of the expression of a mutant form of merlin.\textsuperscript{22} The xenograft model was created using human schwannoma tissue implanted into the flanks of immunocompromised mice. In the transgenic model, G47Δ was injected once with $10^5$ pfu. The mean reduction in tumor volume 20 days after injection was greater than 75% compared with the control group in which the tumor size either enlarged or remained unchanged.\textsuperscript{26}

In the xenograft model, Messerli and colleagues\textsuperscript{26} first investigated infectability with oncolytic HSV. After inoculation with G47Δ at $10^5$ pfu, histological studies and staining for HSV antigens were performed at 1 day and 2 months postinjection. These tests confirmed that the schwannoma architecture was preserved in this model and that HSV infection had been established and persisted at these time points. Mice in the xenograft model received two inoculations with G47Δ ($10^5$ pfu/tumor) on Day 0 and Day 7. Over a 6-week period, a mean tumor reduction of 40.6% was seen in the directly treated group.\textsuperscript{26} Moreover, contralateral, uninjected tumors also showed a comparable reduction in size, while the tumors in the control group enlarged.\textsuperscript{26} This paper demonstrated a model for the subcutaneous implantation and study of human schwannomas. In addition, it demonstrated the ability of G47Δ to act as an oncolytic agent in schwannomas.

Mähler and associates\textsuperscript{29} investigated the effect of cellular RAS activity on oncolytic HSV infection and replication in MPNSTs. They tested both G207 and hrR3. A mutant derived from HSV wild-type strain KOS, hrR3 has an inacti-
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vating insertion of E. coli lacZ in the UL39 locus, as in G207. Unlike G207, however, the γ34.5 regions of hrR3 are not inactivated. Theoretically, the presence of fewer gene modifications would enhance the replicative ability of hrR3 compared with G207.

During viral replication, double-stranded RNA is formed, resulting in a host-cell defense mechanism in which PKR is activated. The PKR phosphorylates eIF2α, resulting in a shut off of host protein synthesis. In HSV with intact γ34.5, its product infected cell protein 34.5 binds to protein phosphatase-1α, resulting in the dephosphorylation of eIF2α and the unobstructed continuation of protein synthesis. It is known that the Ras signaling pathway inactivates PKR and enhances HSV replication.

Based on this, Mahller et al. tested the transduction efficiency of G207 and hrR3 and found that at an MOI of 1 the transduction efficiency was greater than 95%. At an MOI of 0.01, transduction was variable and markedly reduced. Next the authors infected MPNST cell lines and measured viral titers. As expected, hrR3 produced higher viral titers than G207. They also demonstrated that, despite oncolytic HSV infection, MPNST cell lines maintain low levels of phosphorylated eIF2α and are susceptible to HSV-mediated oncolysis, particularly by hrR3. Importantly, these authors also demonstrated the attenuated replicative ability of G207 and hrR3 in normal human Schwann cells compared to wild-type strain KOS.

Oncolytic HSV Armed With Transgenes

The use of transgenes to enhance viral oncolysis of malignant peripheral nerve sheath tumors (and to target the stroma and vasculature supporting the tumor) has been demonstrated using a transgene expressing dnFGFR. Fibroblast growth factor receptor signaling dysfunction is thought to play a role in MPNST development and angiogenesis. Moreover, FGFR activates the Ras pathway. In the in vitro model, G47Δ expressing dnFGFR (G47Δ-dnFGFR) demonstrated enhanced efficacy against MPNST lines and vascular endothelial cells. In addition, inhibition of FGFR signaling was demonstrated. Afterwards, examination of viral replication indicated that the transgene did not obstruct viral replication and that replication was more pronounced in proliferating vascular endothelial cells than quiescent cells, the latter emphasizing the safety profile of the virus. In vivo study of G47Δ-dnFGFR showed significant delay in tumor growth compared with controls and a fourfold reduction in vascular structures on immunohistochemical analysis.

Liu and coworkers report the use of another antiangiogenic agent, PF4, as an armed oncolytic HSV. They demonstrated that stable transfection of PF4 into an MPNST cell line and subsequent implantation into nude mice slowed tumor growth compared with controls and that vascularity was reduced in the PF4 group. The transgene was then incorporated into G47Δ, and the resulting virus demonstrated enhanced potency against vascular endothelial cells over G47Δ without PF4. In addition, they showed increased apoptosis and reduced migration of endothelial cells when infected by G47Δ-PF4 without restriction of viral replication. In vivo, they demonstrated increased survival and delayed tumor growth with G47Δ-PF4. A postmortem study of the tumor vasculature revealed a fivefold reduction in the microvessel density of the G47Δ-PF4 group compared with the group injected with G47Δ alone.

Oncolytic HSV and Synergy with Emerging Therapies

A study examining a possible synergy between oncolytic HSV and erlotinib in an intraperitoneal MPNST model was reported in 2007. Erlotinib is an inhibitor of epidermal growth factor receptor tyrosine kinase. Intraperitoneal MPNST xenografts were injected with three doses of either phosphate-buffered saline, G207, or hrR3 at 10⁷ pfu. The authors noted that oncolytic HSV treatment significantly lengthened survival by 20 and 26% for G207 and hrR3, respectively. These authors also examined tumors that survived after HSV infection and demonstrated that they still remained sensitive to the virus but infected virus was no longer detectable in the tumors. They concluded that viral clearance was to blame for limiting the efficacy of the virus. In vitro testing of a combination of erlotinib and oncolytic HSV resulted in enhanced efficacy against MPNST cells compared with either virus or erlotinib alone. However, this effect was not seen in the in vivo model.

Efficacy of Oncolytic HSV in a New Model

In a time of general paucity of schwannoma mouse models to test emerging therapies, Prabhakar and colleagues published their study on creating a mouse schwannoma line and the efficacy of G47Δ in this cell line. In their study, they described the effectiveness of G47Δ on an immortalized human schwannoma cell line and on a mouse schwannoma tumor in immunodeficient mice. The authors demonstrated that G47Δ is able to replicate and cause regression in both tumor types in subcutaneous tumor models in vivo. The human schwannoma line was noted to grow more rapidly but when inoculated with G47Δ, was more susceptible to oncolysis because of the selectivity of HSV to human cells compared with mouse cells and its inherent selectivity for cells that are rapidly dividing. The mouse schwannoma line that Prabhakar and coworkers developed possibly provides an avenue for study of schwannomas in syngeneic immunocompetent mice.

Safety Testing in Human and Nonhuman Primates

The HSV strain F is one of the least virulent wild-type strains when inoculated into the central nervous system. Both G207 and G47Δ, which represent the majority of the work done on peripheral nerve tumors, are derivatives of strain F. In the late 1990s, intracerebral injection of G207 was safely tested in the New World owl monkey Aotus nancymae, a species known to be highly sensitive to HSV infection. In this study, G207 in doses of 10⁴ and 10⁵ pfu was inoculated intracerebrally. No deaths attributable to inoculation with G207 occurred in the primates. A subgroup of primates were reinoculated a year after the initial inoculation and remained healthy for more than 2 years after the second inoculation. In contrast, the control primate injected with 10⁴ pfu of wild-type strain F developed severe HSV encephalitis. Viral shedding and biodistribution of G207 in A. nancymae was reported in 2000. In this study intrac-
erebral inoculation of this species at doses of $10^4$ and $10^5$ pfu was performed. Although anti-HSV antibody levels in serum were raised, neither infectious virus nor viral DNA was detected in saliva, tears, or vaginal secretions. In addition, a postmortem analysis confirmed that G207 DNA was restricted to the brain. This suggests that health care staff and family members are at minimal risk of contacting the virus from shedding.

Markert and coworkers reported a Phase I dose escalation safety trial of G207 in 21 patients with malignant gliomas. The dosing ranged from $10^6$ pfu at one site and culminated at $3 \times 10^6$ pfu at five sites inoculated directly into human brain. There was no evidence of HSV encephalitis, either clinically or at the postmortem examination. Three patients with glioblastomas remained alive when the results were reported and none of the deaths was attributable to G207. Rampling et al. reported similar safety in a clinical trial of HSV 1716, a $y^{34.5}$ mutant, using lower doses. Subsequent to this, Harrow et al. published their study of 12 patients following high-grade glioma resection in whom HSV 1716 was injected into the walls of the tumor cavity, again with no toxicity attributable to the virus.

Conclusions

As the field of genetically engineered viral therapy continues to expand and evolve, a number of factors stand in its favor. The first is its multitude of targets within the array of cancer pathways. In this respect, the use of oncolytic HSV not only exploits our current knowledge of cancer-cell mechanisms but is also helping us to further elucidate these mechanisms. The next factor and important feature is this therapy’s ability to synergize with existing cancer therapies without restricting their efficacy or its own. Finally the safety profile of oncolytic HSV engenders confidence.

Disclosure

Dr. Robert Martuza and Dr. Samuel Rabkin are consultants to MediGene AG, which has a license from Georgetown University to commercialize G207.

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