Oncolytic Herpes Simplex Virus Vectors

Genetically engineered viral vectors are attractive for both gene therapy and direct viral therapy of malignant tumors. These vectors can be highly efficient in the transfer and expression of therapeutic genes and are either naturally tumor-specific with respect to infection or replication or can be genetically modified to attain tumor specificity.

Several modified viruses are being studied for the treatment of human malignancies. Among these, recombinant herpes simplex virus type-1 (HSV-1) vectors have been developed that are replication competent with oncolytic properties but also are attenuated to avoid the development of human disease. These conditionally replication-competent HSV-1 have shown significant efficacy as oncolytic agents in preclinical animal models as well as safety in multiple phase I human trials.

Herpes simplex viruses are large, enveloped DNA viruses with a genome of approximately 152 kilobase pairs. Genetically modified HSV are attractive as replication-competent, oncolytic vectors for several reasons: 1) procedures for constructing novel HSV are well established; 2) multiple genes can be deleted and/or replaced with therapeutic foreign genes without affecting the replication ability of the virus; 3) the biology of HSV and its behavior in humans and nonhuman primates are well studied; and 4) modified herpesviruses can be engineered to retain sensitivity to antiviral drugs already used clinically as a "built-in" safety feature. Also, genes involved in the neurovirulence of HSV-1 are separate and distinct from those conferring oncolytic properties, such that deletion of neurovirulence genes allows selective targeting to glioma cells without loss of oncolytic abilities.

The tumor-targeting mutations that have been engineered into HSV-1 vectors for therapy of malignant glioma are discussed in this article.

Tumor cell killing by genetically engineered HSV, with or without foreign gene inserts, is mediated by at least three mechanisms. First, tumor cells are destroyed as a direct consequence of viral replication restricted to a population of rapidly dividing malignant cells. Second, HSV replication-mediated oncolysis elicits an antiviral immune response as well as immune responses to tumor-specific antigens. Such immune responses are patient- and tumor-specific and might be anticipated to act as an antitumor vaccine with systemic effects. Such responses could play a role in the prevention or control of metastases.

Finally, expression of therapeutic foreign genes that have been engineered into the viral DNA can augment both the oncolytic effects and immune responses. These include cytotoxic "suicide" gene products (e.g., cytosine deaminase or purine nucleoside phosphorylase), antiangiogenic proteins, and specific immune system signaling molecules such as interleukins or chemokines.

To achieve tumor cell-specific viral replication, specific HSV genes are deleted, resulting in an attenuated virus with replication restricted to dividing cell populations. These genes include, but are not limited to, those that encode thymidine kinase (tk), ribonucleotide reductase, or infected cell protein 34.5 (ICP34.5), the $\gamma_1$34.5 gene product. Mutation or deletion of these genes permits the virus to selectively replicate and destroy glioma cells in vivo, without damaging surrounding brain tissue.

A replication-competent HSV-1 with a deletion in its *tk* gene, called *dl*sptk, was the first such mutant to show promise as antiglioma therapy in animals and to show selective replication in neoplastic cells only. However, concerns over the safety of this vector, including histologic evidence of encephalitis at high doses in preclinical models, as well as its lack of sensitivity to standard anti-HSV drugs, prevented its clinical development.

Another conditionally replication-competent, oncolytic HSV with a *lacZ* gene insertion to disrupt U_L39 gene expression has been constructed. U_L39 encodes the large subunit of the viral ribonucleotide reductase, which is essential for nucleotide synthesis in nondividing cells. In the absence of this enzyme, HSV-1 replication is restricted to actively dividing cells, where the virus can use host replication machinery. Because this virus contains a foreign gene insertion, rather than a deletion, the risk of reversion to wild-type by excision of the insert through recombination makes this virus a poorer candidate for testing in human clinical trials.

Modified HSV-1 with deletions of both copies of the $\gamma_1$34.5 gene has shown significant efficacy against brain malignancies in preclinical animal models and safety in phase I human trials in both the United States and Great Britain. $\gamma_1$34.5 encodes two independent

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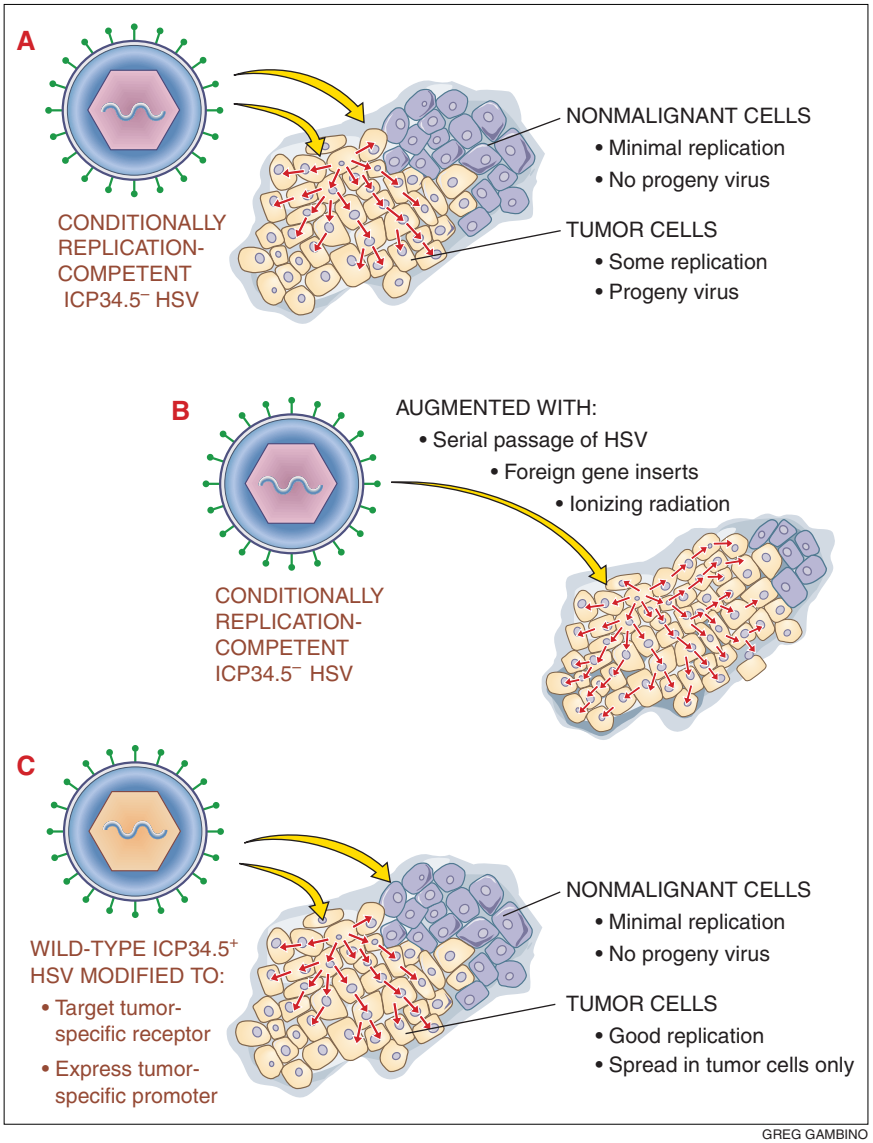

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Tumor cell killing by oncolytic HSV can involve three strategies:

A, Conditionally replication-competent HSV (e.g., ICP34.5⁻) are able to infect and replicate in tumor cells (*yellow*) but not in nonmalignant cells (*purple*). The red arrows show HSV spread to other tumor cells.

B, Tumor cell killing by conditionally replication-competent HSV can be enhanced by serial passage of HSV to select mutants with increased replication and immune evasion properties; by combination with low-dose ionizing radiation to the tumor; or by introduction of foreign gene inserts, including suicide genes or immune-modulating molecules that enhance the antitumor immune responses.

C, Modified wild-type HSV are being developed that specifically target tumor cells either through tumor cell-specific surface receptors or through control of viral virulence genes by tumor-specific promoters.

functions during HSV-1 infection: One of these enables viral evasion of an innate host antiviral response, via the shutoff of host protein synthesis. The second function allows viral replication in neuronal cells. Recombinant HSV-1 that contain mutations or deletions in the $\gamma_1$34.5 gene are unable to replicate in nondividing cells and are only able to replicate efficiently in cells that have defective innate antiviral responses, as is the case for many tumor cells.

The results of three phase I safety trials using oncolytic HSV vectors with deleted $\gamma_1$34.5 genes in patients with malignant gliomas have been reported in the last few years. In the United States, G207, a $\gamma_1$34.5-deleted HSV that also contains a mutation (*lacZ* gene insertion) in the U_L39 gene, was assessed and demonstrated to be safe at doses of up to 3 x 10^9 viruses. No adverse effects attributable to virus inoculation or replication were reported. A phase Ib trial of G207 is currently undergoing data analysis at the University of Alabama at Birmingham.

In two trials in the United Kingdom, HSV-1 strain 1716, a $\gamma_1$34.5-deleted HSV derived from wild-type Glasgow strain 17, was tested in patients with high-grade gliomas for dose-dependent safety and virus replication/survival within the tumor. HSV 1716 was safely administered at doses up to 10^5 viruses, with data supporting viral replication in 2 of 12 patients, and HSV DNA detected in 10 of 12 patients by PCR.

The use of oncolytic HSV is not restricted to malignancies arising in the brain. HSV 1716 also has been safely administered in multiple doses into subcutaneous nodules in patients with metastatic melanoma. Viral replication was detected in the injected nodules by immunohistochemical staining, and no toxicities were observed in any patients. Another oncolytic HSV, NV1020, has completed a phase I safety trial as therapy for liver metastases from colonic carcinoma. A phase I/II trial with this virus has just been initiated which will continue to evaluate safety as well as tolerability and efficacy.

These studies emphasize that attenuated replication-competent HSV vectors are safe for administration to humans, even at high doses.

The safety of genetically engineered, replication-competent HSV as therapy for malignancies has been established in multiple human trials. Clinical trials are currently limited to phase I and II, and at this time, no information is available regarding efficacy in humans. Evidence of viral replication in some treated tumors is promising, but even in preclinical testing in animal models, not all of the animals treated with virus were cured of their tumors.

The ultimate goal is to achieve maximum viral replication specifically in tumor cells with little or no replication in surrounding normal tissue. Two approaches are being investigated to attain this goal. The first strategy is to enhance tumor cell killing mediated by $\gamma_1$34.5-deleted or other conditionally replication-competent HSV, while retaining safety in surrounding tissue. The second strategy is to target replication of wild-type HSV specifically to tumor cells.

For the first strategy, $\gamma_1$34.5-deleted HSV have been serially passaged in human tumor cell lines to select for viruses with enhanced replication ability in tumor cells. In one such virus, which retained its aneurovirulent phenotype, protein synthesis in infected cells was similar to that seen with wild-type HSV, despite the absence of the $\gamma_1$34.5 gene product.

A second deletion was identified in the unique short domain of this virus that altered the expression kinetics of the viral protein Us11. Normally a late gene product, Us11 expression in this modified HSV occurs much earlier in the life cycle. The mechanism by which Us11 interferes with the host innate antiviral response is distinct from that mediated by the $\gamma_1$34.5 gene product. As a consequence, increased viral replication is observed in tumor cells infected with this passaged virus.

In separate studies, another virus serially passaged in tumor cells was selected that showed wild-type protein synthesis despite the $\gamma_1$34.5 deletion; however, this virus also had partially restored neurovirulence. The second site mutation has not yet been identified in this virus. Other studies are in progress to identify additional mechanisms by which the host blocks viral replication and to subvert these antiviral responses.

Replication and tumor cell killing of $\gamma_1$34.5-deleted HSV can be enhanced when combined with radiation therapy. Final approval is pending for a phase I clinical trial combining irradiation with G207 therapy to determine the safety of this approach.

Finally, $\gamma_1$34.5-deleted HSV have been constructed to express therapeutic foreign genes. One example of this approach is the development of oncolytic HSV that express naturally cytotoxic "suicide" gene products, such as cytosine deaminase and purine nucleoside phosphorylase. These HSV are not only capable of direct tumor cell killing, but also generate toxic compounds (i.e., cytosine deaminase converts 5-fluorocytosine to toxic 5-fluorouracil) which kill adjacent uninfected tumor cells (bystander effect).

HSV also have been engineered to express immune signaling molecules. Some examples include $\gamma_1$34.5-deleted HSV that express interleukin (IL)-12 or IL-4, which have been used to treat brain tumors in mice. The expression of interleukins during viral infection stimulates an immune response with enhanced tumor cell killing when compared with $\gamma_1$34.5-deleted HSV that lack foreign gene inserts.

For the second strategy, tumor-specific replication of wild-type HSV can be achieved either by targeting HSV through tumor cell-specific receptors or by controlling $\gamma_1$34.5 gene expression with a tumor-specific promoter. In a proof of principle study published last year, the gD surface glycoprotein of HSV was modified to express a chimeric glycoprotein into which IL-13 was inserted (gD-IL13). The chimeric protein re-targets the virus to infect cells expressing the IL13Rα2 receptor, an altered cell surface receptor that is overexpressed by nearly all glioma cell lines. These were the first data showing that the HSV gD glycoprotein could be altered to use another cellular protein as its receptor and thus allow targeting of HSV vectors to specific cell types.

In summary, phase I clinical data have shown the safety of genetically altered, oncolytic HSV for cancer therapy. Phase II/III clinical studies of these novel viruses will determine their efficacy. Because many tumors, including malignant gliomas, are a whole organ disease, current efforts are directed at developing tumor-specific therapies that can seek out and destroy all tumor cells, even at sites remote from the primary tumor bed. Finally, effective delivery of these vectors remains a critical subject for future investigations.

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