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Strategies and Clinical Trials of Oncolytic Herpesvirus and Adenovirus in Treatment of Cancer

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In the search for novel strategies, oncolytic virotherapy has recently emerged as a viable approach to specifically kill tumor cells. Unlike conventional gene therapy, it uses replication competent viruses that are able to spread through tumor tissue by virtue of viral replication and concomitant cell lysis. Recent advances in molecular biology have allowed the design of several genetically modified viruses, such as adenovirus and herpes simplex virus that specifically replicate in, and kill tumor cells. An overview will be given of the general mechanisms, strategy, and clinical trials by which oncolytic HSV and adenovirus achieve tumor cell-specific replication and antitumor efficacy.

Oncolytic Adenovirus

One of the most extensively studied viruses that has been genetically engineered for oncolytic purposes is adenovirus, in particular serotypes 2 and 5. In order to replicate, adenoviruses promote entry into the cell cycle of the host cell through expression of the E1A and E1B. E1A competes with E2F for binding to pRb, resulting in release of transcription factor E2F and subsequent transition from G1 into S phase. In addition, E1B-55kd is able to bind and inactivate p53. If it were not for E1B-55kd expression, cellular levels of p53 would rise in response to adenoviral infection with resulting apoptosis or cell cycle arrest attenuating viral replication. Nearly all tumors possess defects in their p53 and/or pRb pathways. Thus, E1A and E1B gene products become dispensable in these tumor cells, as E1A- or E1B-defective adenoviruses can still replicate in pRb- or p53-defective tumor cells, respectively.

The first replicating-competent adenovirus, ONYX-015 was reported in 1996. ONYX-015 is a type 2, type 5 chimeric adenovirus that contains alterations in the E1B gene. It was demonstrated that ONYX-015 efficiently replicated only in cells lacking functional p53 and it caused significant tumor regression in mice bearing human tumor xenografts, due to tumor-specific viral replication. The safety and antitumor efficacy of ONYX-015 have been tested in numerous phase I and II clinical. However,

other clinical trials were less promising. With i.t. treatment of pancreatic tumors or ovarian carcinoma no objective tumor responses were observed.

Another strategy that has been used to make adenoviruses tumor selective is by mutation of the E1A gene. E1A mutant adenovirus, *dl*922-947, with a small deletion in conserved region 2 (CR-2) of E1A was shown to replicate specifically in cancer cells with abnormalities in the pRb-pathway and to reduce the incidence of metastases in a breast tumor xenograft model following intravenous injection. These E1A virus mutants have not yet been tested in clinical trials, but are more promising as antitumor agent than ONYX-015, since *dl*922-947 demonstrated significantly greater potency compared to ONYX-015 both in vitro and in vivo.

Oncolytic HSV

Besides adenovirus, herpes simplex virus 1 is one of the most extensively studied viruses for oncolytic virotherapy. HSV-1 is an enveloped, double-stranded DNA virus with a genome size of approximately 152 kb and is generally neurotropic. In first Oncolytic HSV termed *dl*sptk, the thymidine kinase gene (HSV-tk) was deleted, which is normally needed for nucleic acid metabolism. For its replication, this mutant is dependent on endogenous levels of this enzyme expressed in tumors. Accordingly, *dl*sptk replicated selectively in glioma cell lines and i.t. administration caused growth inhibition of U87 human glioma in both nude and immunocompetent mice. However, apart from infection of tumor cells, healthy neurons were also infected and neurotoxicity was found. For safety reasons, investigators have come up with other HSV-1 mutants that are still sensitive to antiherpetics and show less neurovirulence.

One such mutated virus harbors mutations in both γ34.5 loci. The protein product of γ34.5 blocks the shut-off of host protein synthesis in infected cells by interacting with cellular phosphatase 1 (PP1) to dephosphorylate eIF-2. This leads to production of more progeny viruses from each infected cell. Due to specific deletions in this gene, these HSV mutants are strongly attenuated for their replication in normal cells, like neurons, leading to loss of their neurovirulence. However, γ34.5 mutated HSV-1 replicated in actively dividing cells, like tumor cells. Another genetically engineered virus has been produced, that contains mutations in the ICP6 gene, normally encoding the large subunit of ribonucleotide reductase, needed for generation of deoxyribonucleotides for DNA synthesis. ICP6-mutated viruses replicate only in actively dividing cells that provide ribonucleotide reductase in complementation, rendering them tumor selective. This ICP6 mutant virus showed significant inhibition of tumor growth after i.t. administration in mice bearing human glioblastoma tumors or colon carcinoma.

More recently, herpes viruses have been engineered that contain double mutations. A promising HSV-1 mutant is G207, which has deletions in both γ34.5 loci and an inactivating insertion in the ICP6 gene. G207 exhibits tumor cell-specific replication and antitumor efficacy in both in vitro and in

vivo models of malignant gliomas, colorectal cancers, ovarian cancer, breast cancer and prostate cancer. A phase I clinical dose escalation study of G207 in the treatment of recurrent malignant glioma was initiated and no toxicity was found, even at the highest doses. NV1020 is another genetically engineered oncolytic herpes virus that contains a deletion in the endogenous HSV-tk gene as well a deletion in one of the two γ34.5 genes. NV1020 was tested for its oncolytic potency in preclinical models of pancreatic carcinoma, transitional cell carcinoma, head and neck squamous cell carcinoma, hormone resistant prostate carcinoma, and epidermoid carcinoma. In these studies, NV1020 replicated selectively in tumor cells in high titers and caused rapid tumor regression. Comparison of NV1020 with G207 revealed that NV1020 is capable of more efficient viral replication than G207 in vitro and that the oncolytic efficacy of NV1020 was superior to G207 at lower concentrations of the virus both in vitro and in vivo. Given these quite promising results, a phase I trial of intrahepatic arterial injection of NV1020 in patients with colorectal carcinoma liver metastases was recently initiated. Despite the promising results obtained with engineered HSV-1 vectors described thus far, it is likely that multimodal approach to eradication of cancer will be more effective.

References

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