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The Current Research on the Use of Oncolytic Herpes Simplex Virus in Treatment of Gliomas and Glioblastomas

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Oncolytic virotherapy uses naturally occurring or modified viruses to lyse cancer cells as well as help activate the host's immune system. Currently, oncolytic virotherapy using many viruses on many tumor types are being researched. The oHSV-1 virus in cancer treatment is of particular interest due to recent success using this virus multimodally with other immunotherapies or by genetically engineering the virus to change certain functions or expressions of relevant genes and biomolecules such as those involving viral replication. The idea of oncolytic virotherapy (OVT) to treat cancer has great potential, but certain problems such as infection of untargeted cells, blocking of the therapy by a host's immune system, toxicity due to therapy, and more do exist. On the other hand, there have been some promising results when OVT is used concurrently with other cancer immunotherapies such as checkpoint blockade therapy (CBT) and adoptive cell therapy. Discussed here are studies on gliomas and glioblastomas being tested concurrently both in vitro and in vivo with genetically engineered forms of the oncolytic herpes simplex virus (oHSV-1) that attempt to improve treatment of glial cell cancer. Of specific interest are the roles of specific biomolecules involved in oHSV-1 therapy, promising forms of genetically engineered oHSV-1, and how to use/manipulate these key molecules to increase tumor specificity, viral replication, and tumor regression.

Introduction

Glioblastomas are malignant tumors arising primarily from astrocytes, the nerve cells of the brain. Primary glioblastomas, also called glioblastoma multiforme, are some of the most common and life-threatening forms of brain cancer as there are no current curative treatments with an average survival of 12-16 months in adults. [2,3,7] For this reason it is the hope that a curative treatment can be found in the treatment of these fatal malignancies. Gliomas are typically benign forms of glioblastomas, but still lead to complications, and although they are slower growing than a malignant tumor, tend to reform once removed. Because of the strong defenses that glioblastomas present during treatment, a multifaceted approach, modified specifically to evade or block these barricades is needed. [1,2] In recent studies oHSV-1 has been shown to be efficacious in the treatment of gliomas and glioblastomas, with concurrent treatment using other immunotherapies as well as genetically engineering specific genes of the oHSV-1 virus showing improved results. oHSV-1 is so successful due to its ability to selectively lyse cancerous cells, activate the innate and adaptive immune system, and its large genome in which there are many candidates open to being genetically engineered and altered (Zhang et al., 2013).

Recent Progress

ICP4 is an essential gene of the HSV-1 virus and is essential to its cellular (viral) replication. The

removal of this gene causes the inability of viral replication. This essential gene can be regulated, rather than deleted, allowing the essential process to be retained. Et al. targeted glioma tumors by triple-regulating the glioma specific HSV-1 amplicon virus (SU4-124 HSV-1). Transcriptional, post-transcriptional, and translational regulation of ICP4, which is essential in viral replication of HSV-1, was achieved by genetically engineering specific components that work together in the regulation and expression of this gene. In modifying this oncolytic virus, the promoter of ICP4 was replaced with survivin, and two regions, 3'UTR and FGF 5'UTR, were added that contained the target sequence for miRNA. These were added to regulate the expression of ICP4 and increase the tumor specificity of the oncolytic virus in treatment and target glioma neoplasms.

(Delwar et al., 2016) used a non-tumor-specific HSV-1 whose ICP4 gene was driven by a generic CMV promoter, CMV-ICP4-HSV-1, as the control virus when testing the effects of SU-124 HSV-1. Through a series of tests (Delwar et al., 2016) found that SU-124 HSV-1 has a strong antitumor effect on glioma cells, tumor specificity is increased using triple-tumor specific regulation, and using SU-124 HSV-1, antitumor responses are increased without harming normal tissue when tested in vivo (Delwar et al., 2016).

Another study conducted by (Fukuhara et al., 2021) focused on ways to improve treatment in slow growing tumors, namely gliomas, while retaining the efficiency in treating fast-growing tumors, specifically glioblastomas. In this study, researchers constructed a new third generation oHSV-1 called T-hTERT using a second generation oHSV-1 called G47Δ, that was made from a first generation oHSV-1 called G207. Through all three genetically modified generations of the oHSV-1, there were specific components of the virus that were modified. G207 is a form of oHSV-1 with a deletion in both copies of the *γ34.5* genes as well as a lacZ gene insertion causing inactivation of the *ICP6* gene. The deletion in the *γ34.5* gene allows viral replication within cancer cells but not within regular cells. Inactivation of the ICP6 gene through insertion of the lacZ gene has a similar effect but only permitting viral replication in dividing or fast-growing cells. The first generation oHSV-1 G207 has proven to be highly specific to cancer cells, but its replication does not compare with the wild type oHSV-1. Then G207 was modified further by adding a further deletion in the *α*47 gene, creating the second generation oHSV-1 named G47Δ. This further deletion in G47Δ of the *α*47 gene enables the oncolytic virus to escape from immune surveillance in the host environment. While G47Δ has proven to be highly specific to cancer cells, as with G207, and while it can go undetected by the immune system, unlike G207, the replication is still inefficient due to the restrictions of the inactivated ICP6 gene that causes the virus to only replicate in fast growing or diving tumor cells, leaving gliomas (slow-growing tumor cells) to grow unnoticed (Fukuhara et al., 2021).

The third generation oHSV-1 created in the study has the lacZ gene and the hTERT promoter driven ICP6 gene as well as the previous deletions in both the *α*47 and *γ34.5*. (Fukuhara et al., 2021) inserted the human telomerase reverse transcriptase (hTERT) promoter controlled ICP6 gene to activate ICP6 in a tumor specific manner. This triple mutated oHSV-1 virus showed promising results.

The third generation T-hTERT oHSV-1 proved to be more effective in two slow growing tumors, OS-RC-2 and DU145, than the control viruses termed T-pro, which contained an intact ICP sequence without the promoter, and T-01, which did not contain the ICP6 gene. It also retained a high efficiency in the fast-growing tumor, U87MG comparable to the control viruses. The safety features of previous oHSV-1 generations were also retained (Fukuhara et al., 2021).

A new cancer immunotherapy called antiangiogenic therapy was utilized by a study using oHSV armed with interleukin-12(IL-12)

and angiostatin in the treatment of glioblastomas. Hypervascularity is a known pathognomic feature of glioblastomas. This means that cancer tissue undergoes a great deal of angiogenesis, growing their own, new blood vessels, and is over vascularized. Antiangiogenic therapy focuses on preventing cancer tissue from growing blood vessels. By decreasing the ability of cancer to grow blood vessels, antiangiogenic therapy can also prevent the growth of the cancer. The two antiangiogenic agents used in this study are IL-12 and angiostatin. "IL-12 is a principal Th1 cytokine with antiangiogenic properties. Its antiangiogenic and antitumor effects were successfully tested when delivered to a mouse squamous cell carcinoma using oHSV as a vector" (Et al.). Angiostatin is similar in its antitumor and antiangiogenic effects. "Angiostatin, an internal fragment of plasminogen, induces regression of the tumor vasculature, inhibits cancer cell invasion, and suppresses tumor growth *in vivo*, including in GBM" (Et al.). Although both angiostatin and IL-12 individually have shown their promise as antiangiogenic agents, both are limited by systemic toxicity and issues in delivery. When expressed together using oHSV-1 in Et al.'s study, in the form of G47Δ-mAngio and G47Δ-mIL12, the combination of IL-12 and angiostatin showed promising results. Both G47Δ-mIL12 and G47Δ-mAngio were used to treat two different intracranial human glioblastoma models. One of the models was from a U87 glioma cell line while the other was from MGG4, a cancer stem cell line. When the armed oHSVs were used to treat these tumor cells lines, increased angiogenesis resulted, both in vitro and in vivo, supporting the author's hypothesis that" a combination of oHSVs armed with angiostatin and IL-12 could improve GBM treatment" (Zhang et al., 2013).

(Zhang et al., 2013) also took immunohistology analyses of the tissues that had been treated with G47Δ-mAngio and G47Δ-mIL12 which shed some light of the mechanisms of antiangiogenic therapy. From these analyses (Zhang et al., 2013) found that treatment with the two-armed oHSVs decreased the expression of angiogenic markers to a greater degree than either of the viruses when used alone. It was also found that a decrease in angiogenesis leads to a decrease in macrophage infiltration and therefore increased viral spread throughout the tumor (Zhang et al., 2013).

Discussion

By analyzing results of the current research reported above, it is clear to see that the use of OVT as a treatment for cancer is only just beginning. All the studies reported above showed promising results when using oHSV-1 in the treatment of gliomas and glioblastomas. Concluded from the studies above, there are many ways in which oHSV-1 can be manipulated, armed, and engineered to improve its usefulness in treating tumors originating in the glial cells. Through the different modifications to oHSV-1 reported above, many aspects of oHSV-1's treatment abilities were improved such as intratumoral viral replication, cancer cell specificity, treatment of slow growing gliomas, and ability to evade the host immune system. The key components of oHSV-1 that were genetically modified to improve the virus were the ICP6, α47, γ34.5, ICP4 genes, the hTERT and surviving promoters and the antiangiogenic agents, IL-12 and angiostatin. While there is currently only one widely approved OVT called T-VEC, or Imlygic, which is a modified oHSV used in the treatment of melanoma patients, there are many other OVT therapies currently in clinical trials. Some of these include adenovirus, vaccinia virus, reovirus, and other modified forms of herpes simplex virus that will no doubt prove to be very useful in the future treatment of many cancer types (Oncolytic virus therapy – CRI, 2023). Something that is currently not clear is the accessibility of these future oncolytic virus therapies. As of 2015, when Imlygic was approved for the treatment of melanoma patients the average cost was estimated to be 65,000 dollars (FDA approves first oncolytic virus therapy: Imlygic for... : Oncology Times, 2015). This does not seem to be an accessible price point

for most of the population and could turn out to be a substantial roadblock for many patients. Perhaps soon as more OVTs are approved, this price point will be lowered, increasing the accessibility of OVTs as a common and effective cancer treatment.

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