COMMENTARY

Viral therapy of glioblastoma multiforme

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Of all human malignancies, glioblastoma multiforme (GBM) is among the most refractory to existing therapies despite combination approaches of surgical resection, irradiation, and chemotherapy. The median survival is ~12–14 mo, regardless of the therapeutic intervention. The 5-y survival of patients with a diagnosis of GBM is less than 9.8% (1). This remarkably poor rate of survival begs the question of why our interventions have not been more beneficial over the last several decades. Cheema et al., in PNAS (2), discuss a unique multifaceted approach to treatment of glioblastoma.

Challenges of GBM

Primary intracranial malignant gliomas, once thought to arise from the most numerous cell type in the brain, the astrocyte, now are felt by most experts to arise from glioma stem (or progenitor) cells. Due to an accumulation of unique biologic properties, these tumors pose significant challenges for successful intervention. They have heterogeneity, both morphologically and genetically, among and within tumors. There is significant neovascularization, and the tumors are highly invasive. The heterogeneity of these tumors is reinforced by the observation that GBM stem cells (GSCs) can be isolated from human tumors and provide evidence of differentiation into multiple, more mature lineages. The GSCs can lead to self-renewal and proliferation (3, 4). These cells have an inherent resistance to radiation and are believed to exclusively maintain the neoplastic clone. Several studies have suggested that the resistance of GBMs to current chemotherapy and radiation is mediated by cells of GPC lineage (5–7). A second characteristic, neovascularization, is a signature of malignant gliomas. Likely, a critical biological mediator of neovascularization in glioma is VEGF, up-regulated in part due to the significant hypoxia present in the tumor microenvironment. A third characteristic of GBMs is their invasiveness, providing one of the most frustrating hurdles to the successful development of therapy. Migration of glioma cells occurs predominantly along white matter tracks and along perivascular spaces. Evidence of the importance of this property is that, before the advent of antiangiogenic therapies, the majority of malignant gliomas recurred within 2 cm of the margin of treated disease despite "clean" surgical margins by imaging.

Approaches to Treatment

The tumor itself is associated with a degree of immunosuppression, be it mediated by the tumor microenvironment, increased expression of transforming growth factor-β, IL-10, and prostaglandin E, and their impact on the systemic host responses. All of these factors taken together have led to aggressive efforts to develop novel approaches to the management of GBMs. One such novel approach is the use of oncolytic herpes simplex viruses (oHSV) for experimental therapy by direct administration into the tumor bed. The founding premise of virotherapy is that genetically engineered HSV has a decreased capacity to produce encephalitis. These viruses are defined as having decreased neurovirulence. These engineered viruses lack the ability to replicate in normal, postmitotic neurons but retain the ability to replicate in and potentially destroy other types of tissue, including gliomas and other brain tissues. These neuroattenuated HSV constructs that have been studied to date are, for the most part, deleted in the diploid $\gamma_1$34.5 gene. This gene maps in the inverted repeats of the unique long segment of HSV. The phenotypic property of neurovirulence maps across the entire gene. Its deletion reduces neurovirulence of WT HSV by more than five logs (8). Furthermore the biology of these viruses indicates an impaired ability to establish latency and be reactivated from ganglionic tissue in murine, rabbit, and guinea pig models (9). In anticipation of clinical trials, numerous animal models of gliomas have demonstrated that both an oHSV deleted with both copies of $\gamma_1$34.5 and oHSV that expressed foreign genes, particularly cytokines, can prolong survival in murine models of GBM. Indeed, second-generation oHSV that expresses either murine or human IL-12 has prolonged survival and enhanced the number of animals surviving compared with the first-generation virus, G207, a construct that has been studied in human trials (10, 11).

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Of relevance to the development of new experimental oHSV therapeutic approaches to GBMs is the documentation that the first generation virus, G207, has been successfully evaluated in several phase 1 studies. Each of these studies demonstrated safety, as no patient developed evidence of encephalitis. Furthermore, in select patients, survival appeared to be enhanced, with MRI evidence of tumor response.

Each of these observations sets the stage for the study by Cheema et al. (2). To summarize, the tumor microenvironment, particularly as it relates to the potential contribution of immune suppression, heterogeneity of cells, and, potentially, GSCs, underscores the nature of the problem. Previous studies have suggested that GSCs are susceptible to treatment with oncolytic HSV (12, 13). Until this study, syngeneic models of GSCs were sparse, and none that were suitable for examination of oHSV existed. The current model makes use of a glioma line with high basal expression of GSC markers when grown under appropriate conditions and then examined in syngeneic immunocompetent C57BL6 mice. Following the establishment of tumors, the tumors were found to express characteristic stem cell markers such as nestin, mProminn (the CD133 homolog), and Olig2. With the establishment of the syngeneic model, the investigators used an oHSV deleted in both copies of $\gamma_1$34.5 and ICP47, a protein that blocks MHC class I presentation in human cells. This virus also expresses murine IL-12. The expression of IL-12 in this vector is exceedingly important as it demonstrated both

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antiangiogenic effects and induction of a Th1 immune response, with partial inhibition of the T-reg response. The available data demonstrate prolonged survival and decreased tumor growth following inoculation of this virus in two divided doses into the tumor bed itself. Such studies are extremely relevant in terms of understanding the biology of GSCs because of the availability of an appropriate syngeneic murine model, which the authors have gone to great length to validate and have characterized the nature of the antitumor response in this model.

These studies bring further attention to the relevance of the expression of IL-12 in oHSV. Soon a human trial will be initiated, administering an oHSV that expresses human IL-12 directly into the GBM tumor bed. It is hoped that this approach, as with the approach of the Δ47γ-mIL-12 virus, will provide further advances in the management of patients with GBM. Clearly, the potential advantage of an agent using multipronged approaches (direct oncolysis, induction of an antitumor T-cell response, and development of an antiangiogenic response) will be critical in malignant glioma—a tumor that is highly diverse, with heterogeneity both across patients and within a single tumor. Likely, further combinations of approaches will be required in the development of future such therapies, including the use of multiple cytokine-expressing oHSV platforms, radiation, and chemotherapy.

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