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A murine model for virotherapy of malignant brain tumors

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Abstract

Glioblastomas (GBMs) are very aggressive and almost incurable brain tumors. The development of new therapeutical approaches capable of selectively killing cancer cells could represent a step forward to fight cancer. With this aim we tested the efficacy of a novel oncolytic therapy based on recombinant herpes simplex viruses (HSVs) infecting exclusively cells expressing the human receptor HER-2 [1, 2], overexpressed in about 15% of GBMs [3]. For this study we exploited a murine GBM model based on PDGF-B embryonic transduction [4, 5]. We engineered cell cultures derived from this model to express HER-2 and we injected intracranically such cultures in NOD/SCID mice. We evaluated the efficacy of R-LM113, a recombinant HSV directed to HER-2, in this glioma model expressing HER-2. We demonstrated that mice injected with engineered glioma cells infected with R-LM113 developed gliomas with a statistically significant delay compared to mice injected with non-infected engineered glioma cells.

Introduction

GBMs are tumors with generally poor prognosis. Their great infiltrative ability makes surgical therapy generally ineffective and furthermore their chemo- and radio-resistance protects them from these therapeutic approaches.
An effective therapeutic approach should discriminate between normal and cancer cells and reach individual cells

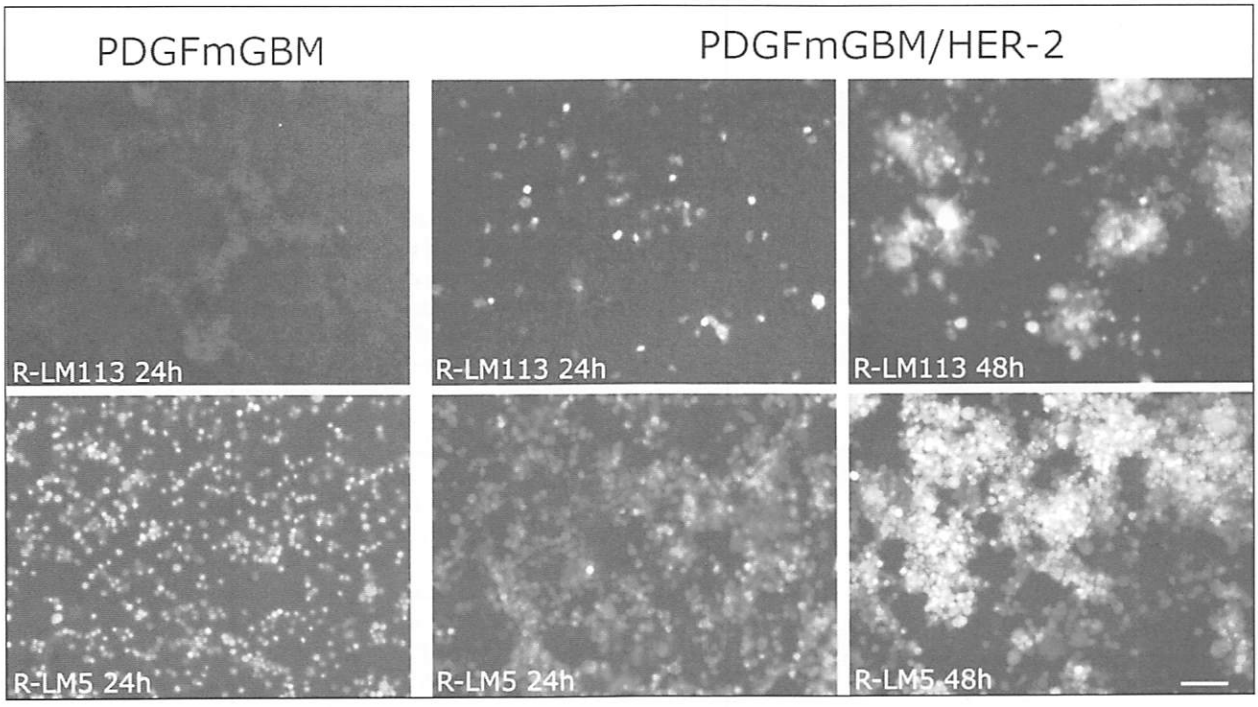

Figure 1. Microphotographs show the GFP-fluorescence of the indicated cell cultures infected by the two different recombinant HSVs. The left panel shows that PDGFmGBM are susceptible only to R-LM5 with wild-type tropism. The right panel shows that HER-2 engineered cultures are infected also by the retargeted R-LM113, which spreads within the time. Scale bar 100 μm.

that migrated away from the tumor mass. A possible way to selectively kill cancer cells is to exploit the differential expression of membrane receptors to provide a portal of entry to oncolytic viruses with a reprogrammed tropism. An example of these recombinant viruses is R-LM113, a herpes simplex virus fully retargeted to the human receptor HER-2, and detargeted from its natural receptors [1].
To test this approach against a brain tumor in vivo, we engineered with HER-2 a murine model of GBM we previously developed (PDGFmGBM) [4, 5]. These tumors are similar to human GBMs and are highly tumorigenic when transplanted in the adult mouse brain [4, 5], therefore they are the ideal model to test the efficacy of this oncolytic therapy.

Materials and methods

PDGFmGBM cultures were maintained in DMEM-F12 added with B27 supplement, bFGF and EGF and plated on to Matrigel-coated flasks [5]. PDGFmGBM cells were stably transfected with the pcDNA3/HER2 plasmid and then checked for HER-2 expression. Immunostainings were performed using a mouse monoclonal antibody against HER-2 which was revealed with secondary FITC–conjugated antibody. Cells were injected in deeply anesthetized adult animals mounted on a stereotaxic apparatus [4].

Results

PDGFmGBM cultures were transfected with a plasmid carrying the coding sequence of HER-2 and a clone stably expressing HER-2 at the cell plasmamembrane was selected and designated as PDGFmGBM/Her2.
As expected, HER-2 expression did not affect the tumorigenicity of the clone which, when intracranically transplanted, generated secondary tumors with the same onset-time and histopathological features of PDGFmGBM cells. We then examined R-LM113 for its ability to selectively infect PDGFmGBM/Her2 cultures and for its spreading potential. We demonstrated that the virus is able to infect and spread only in PDGFmGBM cells expressing HER-2. On the other hand, a recombinant HSV with natural tropism (R-LM5) [1] infects and spreads in both cell types (Figure 1).
Finally, we assessed the therapeutic efficacy of the R-LM113 (Figure 2). We intracranially injected NOD/SCID mice with PDGFmGBM/Her2 cells pre-infected with R-LM113. In parallel, as control, an equal number of mice was injected with PDGFmGBM/Her2. While control mice died with a median of 55 days, mice injected with PDGFmGBM/Her2 cells pre-infected with R-LM113 developed tumors with a median of 119 days (log-rank test $p<0.005$).

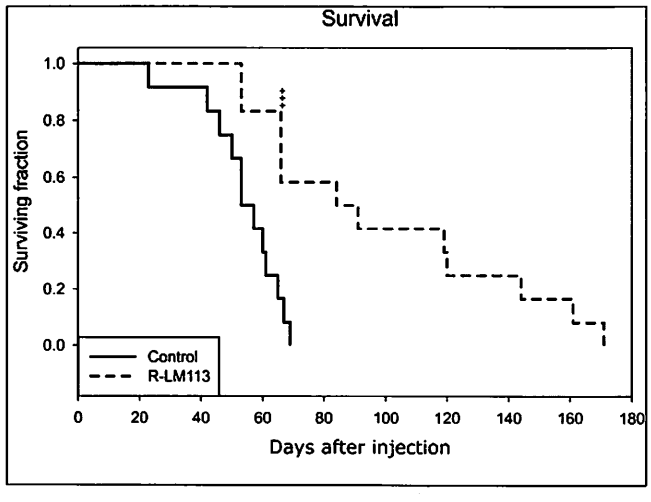

Figure 2. Survival curves for mice transplanted with PDGFmGBM/Her2 cells infected with R-LM113 (dotted line) or not infected (black line)

Discussion

The proof of concept for the efficacy of a virotherapic approach in intracranial tumors could represent a crucial step forward in the global effort to discover novel anti-cancer agents able to selectively target GBM cells. In our attempt to develop a GBM murine model suitable to test the antitumor potential of a HSV retargeted to HER-2, we engineered PDGFmGBM cultures to express HER-2. Our data shows that the R-LM113 virus efficiently infects HER-2 expressing cells but is not able to infect and spread in the non-engineered PDGFmGBM cultures. Moreover, R-LM113 is safe *in vivo*, as it did not induce encephalitis when injected in the brain of NOD/SCID mice.
Finally, the virus was able to significantly reduce tumor aggressiveness, nearly doubling mice survival. It will be important to evaluate whether survival can be prolonged even when the retargeted HSV is administered to established tumors.

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