ONCOLYTIC VIRUSES IN THE TREATMENT OF HIGH GRADE GLIOMAS

Direct oncolysis & activation of antitumor immunity

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Oncolytic virotherapy (OVT) is a promising novel approach in the treatment of Glioblastoma Multiforme (GBM). GBM is a WHO grade IV neoplasm and the most frequent brain tumor in adults, representing over 50% of all primary brain tumor cases[1]. Despite multimodal treatment the prognosis of GBM patients remains dismal with a median survival of 14.6 months[2]. Relapse is universal and prognosis for relapsed patients is even worse[3]. OVT has the potential to lower the tumor mass considerably through direct cancer cell killing, as well as to alter the immunosuppressive microenvironment within the tumor. Several oncolytic viruses have been shown to induce antitumor immune responses, comprising both the innate and the adaptive arm of the immune system[4]. Because of its tumor debulking capacity, OVT holds great potential for patients unable to undergo (total) surgical resection of their tumor. Diffuse intrinsic pontine glioma (DIPG) deserves special attention in this respect. DIPG is by far the most common and most aggressive brainstem glioma. It occurs exclusively within the pediatric population and has an extremely poor prognosis, with 100% mortality within two years[5]. DIPG falls amongst the most difficult pediatric lesions to treat as surgery is not an option (based on the anatomical location in the pons and the highly diffuse nature of the tumor), chemotherapy has shown no benefit and radiotherapy leads to only a transient improvement in symptom free survival[6]. In this project we aim to evaluate the potential of 3 oncolytic virus strains; reovirus, parvorius and Newcastle disease virus (NDV), in *in vitro* and *in vivo* models of high grade glioma (HGG) and DIPG. So far, we have demonstrated in an orthotopic, immunocompetent HGG mouse model that NDV and reovirus treatment significantly prolong median survival and induce long term survival in a fraction of treated animals. The effect on tumor growth was visualized by MRI. We have further shown that this therapeutic effect is, at least in part, immune mediated, as it was abrogated in $Rag2^{-/-}$ mice lacking B and T cells. Immunocompetent tumor-bearing animals surviving long term after OVT were able to resist a second tumor rechallenge. Ongoing work is aimed at further investigating this antitumor immune response. *In vitro* we have assessed the cytotoxic effect of all three viruses on HGG (GL261, E98, U373, U87) and DIPG (NEM157) cell lines. These experiments demonstrated sensitivity to virus-mediated cell death in all cell lines tested, albeit to a different extend. Flow cytometry and western blot analyses have revealed that all 3 virus strains induce apoptosis in the NEM157 cell line. Apoptosis induction was not seen in the E98 cell line, suggesting another mechanism of cell death to play a role in the virus-mediated cytotoxicity. The *in vivo* E98 DIPG model has been established and the tumor growth visualized by MRI[7]. In ongoing work we focus on OVT in DIPG *in vivo*, as well as on combining OVT with our established immunotherapy approach in the HGG mouse model[8].

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