ENHANCERS AND REPRESSORS OF IMMUNOTHERAPY: TRANSLATIONAL PERSPECTIVES ON GENE-MEDIATED CYTOTOXIC IMMUNOTHERAPY IN GLIOBLASTOMA

Background Gene-mediated cytotoxic immunotherapy (GMCI) is a local tumor immunotherapy that uses agalitimagene besadenovec (a non-replicating serotype 5 adenovirus, expressing HSV1 thymidine kinase) with the promod ganciclovir to induce DNA double strand breaks (DSB), leading to immunogenic tumor cell death and intratumoral immune cell invasion. Here we investigate potential repressors and enhancers of GMCI’s effectiveness. GMCI is currently in clinical trials in combination with immune checkpoint blockade in glioblastoma. Thus we set out to identify potential areas to improve this approach for future application. Dexamethasone is used in symptomatic treatment of glioma patients, although it is known to cause immune suppression. However, the influence of dexamethasone on the efficacy of GMCI has not been explored. In contrast, DNA damage response inhibitors like the ATR inhibitor (ATRi) AZD6738 might not only ameliorate the cytotoxic but also the immunogenic profile of GMCI, rendering it an attractive combination partner.

Methods We investigated the effects of ATR-inhibition and dexamethasone on GMCI in vitro using cytotoxicity, flow cytometry and T-cell-killling assays in glioblastoma cell lines. The impact of dexamethasone and ATRi in vivo was assessed in an orthotopic syngeneic murine glioblastoma model. Tumor immune infiltrates were analyzed with flow cytometry.

Results Cytotoxicity assays showed that dexamethasone has a slight impact on GMCI in vitro. In T-cell-functional assays, we observed a significantly impaired tumor cell killing. Immune cell response assays revealed a reduced immune cell proliferation after co-culture with supernatant from dexamethasone or combination treated glioblastoma cells. In vivo, while treatment with GMCI alone resulted in longer median symptom-free survival (39.5d) versus no treatment (23d), the combination of GMCI and dexamethasone resulted in the significant reduction of this effect (29d vs 39.5d; p = 0.0184).

The combination of ATRi with GMCI proved to be synergistic in cytotoxicity assays. Flow cytometry revealed a significant increase in DSB-associated H2AX foci as well as an improved immune profile by downregulation of GMCI-induced PD-L1 expression. In vivo, the combination with ATRi led to an increase in long-term surviving animals (66.7%) compared to GMCI (50%) and proved to be highly significant compared to the untreated control (p=0.0022).

Conclusions Our data suggest that dexamethasone may decrease the efficacy of immunotherapy for glioma through impaired T cell function; this emphasizes the need in identifying alternatives to dexamethasone to prevent attenuated responses in immunotherapies. The combination of GMCI with ATRi however points to additional therapeutic benefit through enhanced cytotoxic efficacy, improved immunogenicity in vitro and increased long-term survival in vivo, making it a promising future approach for the treatment of glioblastoma.

Acknowledgements This was supported by NCI P01CA069246 (Chiocca)