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Characterization of the tumor microenvironment after combined radio- and immunotherapy in a murine glioblastoma model

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Recent therapeutic approaches for glioblastoma (GBM) focus on GBM cells eradication by local radiotherapy (RT) and targeting the tumor microenvironment (TME), e.g. by excluding myeloid-derived suppressor cells (MDSCs) by blocking the CXCR4/CXCL12-signal axis. The effect of RT combined with antagonization of CXCL12 (SDF-1) by a novel Spiegelmer immune modulator on survival, cellular TME and cytokine production was investigated here the first time in an immune-competent murine model as the main objective of the study. Further, the survival effects of a second immune modulator neutralizing CCL2 were evaluated.

Immune-competent 7-12 week-old female wild-type C57BL/6J mice were intracerebrally implanted with $1.5x10^5$ murine GFP-expressing glioma cells (GL261) and survival was monitored up to 100 days. Three different immune modulators (anti-PD-1 antibody (6x 250 µg ip.), CXCL12 and CCL2 antagonists (both 20 µg/g body weight s.c. every 2nd day)) were tested in various combinations (13 groups) with or without half-brain irradiation (1 x 12 Gy). To monitor tumor growth, animals (n = 8-15) were observed weekly via MRI (1 Tesla, T1-weighted) and after a maximum of 100 days, brains were examined for complete tumor regression (MRI, fluorescence signal, immunohistochemistry). To investigate the effects of the combination of CXCL12 antagonist and RT on the immune response, tumor tissue, spleen and blood from animals randomized into four groups (untreated control, NOX-A12, RT, RT with NOX-A12) were analyzed by flow cytometry 20 days after tumor inoculation. Besides, the concentrations of 31 cytokines and chemokines in tumor and plasma were measured by bead-based multiplex immune assay and receptor profiles after RT were determined for the $CD8^+$ effector cells, $CD4^+FOXP3^+$ Tregs and $CD11b^+Gr1^+$ MDSCs. Survival data were statistically analyzed by the Kaplan-Meier method, other data using a one-way ANOVA test or an unpaired t-test with a significance limit of 5% ($p < 0.05$).

As monotherapy, both Spiegelmers had no positive effect on mouse survival, contrary to the anti-PD-1 antibody (32 days median survival (MS), $p = 0.025$). Only the CXCXL12 antagonist combined with anti-PD-1 treatment had an improved survival effect over the control group (42 days MS, $p = 0.003$). RT resulted in a MS of 57.5 days with an overall survival (OS) rate of 25%. Addition of CXCL12 antagonist to RT improved MS to 99 days and overall survival to 50% ($p = 0.19$). CCL2 antagonization combined with other treatments did not result in any survival benefit. Flow cytometry analysis revealed only minor differences in tumor-infiltrating leukocytes and endothelial cells. $CD8^+$ T cells carried exhaustion markers, while a quarter of the $CD4^+$ T cells were regulatory T cells (Tregs). Intratumoral MDSCs ($CD11b^+Gr1^+$) expressing CXCR4 were significantly reduced after RT. Receptor profiling of irradiated MDSCs showed a loss of CXCR4 as single expression or in combination with other receptors. Irradiation leads to a significant increase in receptor combinations $CCR4^+CXCR4^+CXCR7^+$ ($p = 0.01$) and $CCR2^+CCR4+CXCR4^+CXCR7^+$ ($p = 0.04$) on $CD8^+$ effector cells and a slight reduction in $CCR4^+CXCR4^+$ Tregs. Only for CXCL12, an inversion of the gradient between plasma and TME concentration was observed under the influence of the tested immune modulator.

Considering that GBMs are particularly malignant tumor entities, the heterogeneous results under CXCL12 antagonist NOX-A12 as adjuvant to RT warrant further preclinical studies to identify potential impact indicators yet unknown with regard to the suitability of the inhibitor for clinical use.