DUAL TARGETING OF PD-1 AND CTLA-4 SYNERGIZES WITH FOCAL RADIATION TO DURABLY INCREASE SURVIVAL AGAINST GLIOBLASTOMA

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Background The immunogenic role of ionizing radiation raised the use of radiation therapy (RT) as an immune adjuvant in multiple cancers. However, clinical trials testing RT and immune checkpoint blockers (ICB) combination failed to improve the survival of glioblastoma (GBM) patients. We have previously shown that cancer-cell intrinsic type I interferon (IFN-I) is a prerequisite for synergy between RT and ICB in murine breast cancer; a phenomenon that is RT-dose dependent. Therefore, we hypothesized that failure of RT-ICB combinations in GBM is due to the use of a suboptimal RT regimen (i.e. 2Gy x 30). Here, we compared various RT doses and schedules to determine the best immunogenic RT schedule for combination with ICB in murine models of GBM.

Methods First, GL261 and CT2A murine GBM cell lines were used to determine the release of IFN-I related cytokines and the accumulation of double stranded DNA (dsDNA) in the cytoplasm 24h after single (0-20Gy) or fractionated (8Gy x 3 or 6Gy x 5) RT regimens. Next, GBM intracranial tumors were established in syngeneic animals and treated with focal RT (10Gy x 1, 8Gy x 3 or 6Gy x 5). In some settings, mice received anti-PD-1 and/or anti-CTLA-4. Mice were followed for survival or euthanized for flow cytometry analysis. Tumor-free animals were rechallenged with a fresh tumor inoculum.

Results In vitro, fractionationated RT schedules enhanced the content of cytoplasmic dsDNA in GBM cells as compared to single RT doses, with 6Gy x 5 resulting in the highest accumulation of dsDNA. Consistently, GBM cells irradiated with 6Gy x 5 significantly improved the release of IFN-I cytokines as compared to any other RT schedules. In vivo, 6Gy x 5 and 8Gy x 3 RT regimens were superior in controlling GL261 or CT2A growth as compared to 10Gy x 1. The addition of anti-PD-1 or anti-CTLA-4 to the immunogenic RT regimen of 6Gy x 5 did not improve CT2A-bearing mice survival compared to RT alone. However, targeting both PD-1 and CTLA-4 with RT significantly improved survival with 100% of the animals being tumor-free. Tumor rechallenge confirmed the development of protective anti-tumor responses in anti-PD-1+anti-CTLA-4+RT treated animals. Tumor-infiltrating immune cells analysis revealed an increase of T cells in tumors treated with anti-PD-1+anti-CTLA-4+RT, thus reinforcing the role of T cells in this response.

Conclusions While 6Gy x 5 RT combined with either anti-PD-1 or anti-CTLA-4 did not further improve mice survival, dual blockade of PD-1 and CTLA-4 successfully synergized with RT to durably increase survival against GBM. Overall, this study underscores the need to combine immunogenic RT with several ICB to elicit protective anti-tumor immunity against GBM.

REFERENCE

Ethics Approval All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC).