Abstracts

830 TARGETING B CELL SUPPRESSION TO IMPROVE THE EFFICACY OF IMMUNOTHERAPIES IN BRAIN CANCER

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Background Immunotherapy is a promising approach to treat brain tumors, but several factors, especially the suppressive tumor microenvironment (TME), make translation of immunotherapies difficult. Studies in several other solid tumors have revealed the accumulation of germinal-center-like B-cells as a critical survival predictor of immune-checkpoint-blockade (ICB) therapy, suggesting a central of B-cell immunity in driving ICB therapeutic impact.1,2 However, the harsh TME of brain tumors such as glioblastoma (GBM) suppress B-cell activity. We seek to better understand mechanisms of TME-driven immunosuppression and leverage B-cell immunity to enhance immunotherapy effectiveness in brain tumors.

Methods Single-cell and single-nuclei transcriptomic sequencing were used to analyze human GBM and melanoma brain metastasis tumor samples, as well as CT2A murine glioma models. Spatial multiplex analysis and single-cell transcriptomic analysis were used to characterize B-cell interactions within the TME of GBM samples to identify pathways of B-cell immunosuppression.

Results We characterized tumor infiltrating B-cells as activated but suppressed, with expression of co-inhibitory checkpoint molecules such as CD22, CD72, and CD32 that inhibit effector functions such as plasmablast differentiation and antibody production (figure 1). Single-cell transcriptomic and spatial multiplex analysis highlighted the TGF-ß receptor 2 (TGFBR2) as a key regulator of tumor B-cell suppression with tumor associated myeloid cells (TAMCs) and tumor cells being main producers of TGF-ß1 cytokine (figure 2). TGF-ß1 signaling directly inhibited B-cell proliferation and plasmablast differentiation, and induced expression of checkpoint molecules in vitro. Murine and tumor models with conditional knock-outs of TGFBR2 or TGF-ß1, showed significant increased efficacy of PD1 blockade compared to wild type controls (figure 3). The TME of these animals had expanded germinal-center-like proliferating intratumoral B-cells, production of tumor-reactive antibodies, and T-cell activation, and these animals showed increased survival. We also blocked aVß8 integrin, which has tumor-B-cell specificity and activates TGF-ß1, and found that our treatment promoted PD1 blockade efficacy and long-term survival benefit. Combining aVß8 and PD1 blockade eradicated gliomas in nearly 60% of treated mice and promoting immunological memory against tumor rechallenge (figure 4). This robust therapeutic effect depended on B-cell immunity as mice lacking B-cells (B-cell deficiency or intratumoral B-cell depletion using rituximab) failed to prevent tumor growth. After dual treatment, analysis of the TME showed robust cellular proliferation and differentiation into plasmablasts and effector T-cells (figure 5).

Conclusions Our results highlight the importance of B-cells in anti-tumor response, and that promoting Bcell function is a novel approach to boosting the effects of checkpoint blockade in brain tumors.

REFERENCES

Abstract 830 Figure 1 Single cell RNA analysis of Tumor B cells Single cell (sc) and single nuclei (sn) RNA sequencing for inhibitory checkpoint molecules on tumor infiltrating B cells in (A) human glioblastoma (GBM), (B) melanoma brain metastases (MBM), and (C) murine CT2A glioma model. All models show elevated expression of co-inhibitory checkpoint molecules CD22, CD72, and CD32.

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TGFβ1-TGFβ-R2 Pathway Mediates B cell Suppression

(A) Tumor B cells express high levels of TGFβ-R2 in both GBM (left) and MBM (right) samples as well as murine CT2A model. (B) scRNAseq shows that the TME of GBM has high levels of TGFβ1 cytokine. (C) Spatial multiplex analysis shows TAMCs and tumor cells are key producers of TGFβ1 in the TME.

Inhibiting TGFβ Signaling on B cells Increases Survival

(A) We generated conditional knockouts of TGFβ-R2 from B cells. These animals had an improved response to PD1 therapy (p<0.01). (B) Wild type mice with CT2A-TGFβ1KO tumors had improved survival compared to those with CT2A-wild type tumors (p<0.5). B cells in these mice also had improved proliferation and function.

αVβ8 Integrin Blockade Improves PD1 Blockade Efficacy

(A) CT2A bearing mice were treated with dual or single αVβ8 and PD1 blockade. Dual treated animals showed significantly improved survival benefit (n=10 animals/group, p<0.0001). (B) Animals were re-challenged with CT2A tumors in the contralateral hemisphere. Those that also received B cell depletion via rituximab were not able to mount a memory response. (C) Dual αVβ8 and PD1 blockade did not exhibit the same therapeutic benefit in B cell KO mice compared to wild type mice (p<0.01).
Abstract 830 Figure 5  Dual aVb8 and PD1 blockade Improves B cell Functionality

(A) Phenotypic analysis of B cells after treatment show increased proliferation and plasmablast differentiation. (B) Functional analysis of B cells show increased activation and proliferation of CD4 and CD8 T cells as determined via induction of IL17 and granzyme B