TITLE
Regulatory T cells play a key role in VEGF blockade induced tumor immunosuppression

HYPOTHESIS:
the immunosuppressive effect through Tregs is a key factor that inhibits the therapeutic efficacy of the VEGF blockade.

BACKGROUND/AIMS:
Vascular endothelial growth factor (VEGF) has been identified as a critical regulator of angiogenesis and inhibition of this pathway was thought to be one of promising treatment modalities for patients with glioblastoma (GBM) because of it is a highly vascularized brain tumor. Several clinical reports using Bevacizumab (an antibody against VEGF) alone or in combination with other therapeutic approaches have shown some effects, e.g. to prolong recurrent free survival, yet for the overall survival for patients with the primary or recurrent tumors. These results suggest that certain critical suppressive pathways may be activated during the therapy, which jeopardize the antitumor response mediated by the drug. Previous reports demonstrated that VEGF blockade results in vasculatures remodeling, reducing perfusion and increasing hypoxia inside treated tumors, which may lead a metabolic shift and dysfunction of some critical amino acid transporters in tumor cells. Despite the substantial efforts being made in understanding the underlined mechanism of such treatment in rich vesicular tumors, many questions are still need to be answered. GBM tumor microenvironment is an intricate network composing by a dozen of different cellular and soluble components, such as tumor cells; vascular endothelial cells; neural precursor cells; tumor stem cells; stromal cells; residential microglia cells; infiltrating immune cells; cytokines and extracellular matrix proteins etc. Among those components, the vascular endothelial cells and infiltrating immune cells play very important role in manipulating the tumor landscape and affecting tumor progression.
In this study, we focus on the mechanism of failure and the changes of the tumor microenvironment of VEGF blockade treatment in GBM.

METHODS:
1. RNA-Seq analysis of tumor tissues of mice treated with various doses of VEGF blockade shows distinct immune profiling.
2. Single Sample GSEA (ssGSEA) software calculated Regulatory T cell gene-signature score in these samples was found to be increased (not for CD8) in response to escalated dose of VEGF blockade.
3. FACS analysis confirmed the finding at cellular level in the tumors. Immune suppressive markers, such as PD-1, TIM3 and LAG3 were mainly found to be elevated on CD4+ TIL of animals who had failed the treatment of the blockade.
4. Combination of Tregs and VEGF blockades in tumor burden mice showed significantly enhanced antitumor efficacy and prolonged survival were found in animals getting the combination therapy.

RESULTS & CONCLUSIONS

Results:
1. VEGF blockade alters tumor immune landscape
2. A dose-dependent enrichment of Tregs in glioma by VEGF blockade
3. VEGF blockade significantly enhances Tregs in spleen and TIL of tumor burden mice
4. VEGF blockade enhances immune checkpoint molecules on TIL while the animals failed the therapy
5. Tregs depletion prior the therapy restores antitumor reactivates induced by tumor and VEGF blockade

Conclusion:
Regulatory T cells play a key role in VEGF blockade induced tumor immunosuppression