BTRF Grant midterm report

Title of research project: Radiation-immunotherapy combination treatment for glioblastoma: improving the therapeutic ratio

Background: The research conducted in this project will advance our understanding of mechanisms causing immune responses in cancer patients treated with radiotherapy (RT). We anticipate that an understanding of these mechanisms will lead to improvements in emerging therapies that combine radiotherapy with immunotherapy (RT-IO). Importantly, we address ways in which these mechanisms might also cause toxicity in patients undergoing RT-treatment for glioblastoma. Our goal is to define the contributing factors that would improve the RT-IO combination efficacy while reducing its toxicity in glioblastoma, the most common and most aggressive type of malignant primary brain tumor in adults.

Our group and several others recently discovered a molecular basis for RT-induced immune signaling, in which RT-induced DNA breaks leads to the formation of micronuclei (MN). MN formation leads to the activation of cGAS/STING signaling, and inhibiting either the formation of MN or the cGAS/STING pathway impaired interferon signaling. The process of MN formation is poorly understood but is, at least in part, regulated by epigenetic modification of the DNA, specifically by the methylation of to the centromere. This suggests that methylation status may impact cGAS/STING signaling and consequently the RT-induced immune response.

Methylation status is of particular interest in GBM patients. IDH2, a gene that controls the levels of DNA methylation, is frequently mutated in a number of cancers including GBM. Together, these data suggest that the status of IDH2 mutation in GBM might affect their response to RT-IO and may be a useful therapeutic or prognostic biomarker to determine which patients would benefit from RT-IO.

In this project we focused on investigating how IDH2 status may regulate the RT-IO response in cGAS/STING dependent and independent manner.

Progress of the project: A panel of glioblastoma cell lines were genetically modified to ectopically express IDH2 mutant or IDH2 WT. These cells were engrafted in mice and the effect of IDH2 mutation on RT-induced immune response and tumor regression was investigated. We also studied the effect of IDH2 mutation on the formation of MN in response to RT using immunofluorescence. The effect of IDH2 mutation on RT-induced MN formation and inflammatory gene response was also studied in vitro.

Findings summary:

- IDH2 mutation leads to reduction of RT-induced MN formation in vitro and in vivo, the same effect was found when GBM cell lines were treated with DMOG which mimics IDH2 mutation.
- IDH2 mutation affect cGAS activity measured as a function of cGAMP release in vitro.
- IDH2 mutation leads to the reduction of RT-induced inflammatory response,

possibly through the inhibition of cGAS/STING activation via the reduction of MN formation
- The effect of IDH2 mutation on inflammatory response is mainly regulated through cGAS/STING pathway rather than RIG-I pathway
- IDH2 mutation affect tumor response to radiotherapy

Estimated completion time: We are in the process of investigating in more depth the mechanistic pathway through which IDH2 mutation affects RT-induced immune/inflammatory pathway. We are also carrying out extensive in vivo studies to investigate the effect of the mutation on tumor response to immunotherapy and RT/IO combination therapy. This work is expected to be completed by December 2021.

Bibliography of publications:

- Brain Tumor Research Foundation research symposium and student competition, 3rd October 2020, podium presentation.
- Tumor Immunity and Microenvironment (TIME) weekly research seminar, 23rd March 2021, podium presentation.
- Effect of epigenetic regulation on radiation induced toxicity, Review paper, Manuscript in preparation