Effects of combined immune and radiation cancer therapy on measures of anxiety in murine models

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Abstract

Behavioral changes in patients with cancer are multifactorial and may include difficulty concentrating, memory impairment, and increased anxiety. Though behavioral changes are often associated with (neo)adjuvant chemotherapy and/or cranial irradiation, radiation and a pro-inflammatory environment in the brain may also play a role. Novel combinations of systemic immunotherapy and radiation-based neuroimaging (RT) may enhance therapeutic efficacy with regard to the tumor, but how they may modulate behavioral effects is not known. We hypothesize that immunotherapy will modulate the effects of RT on behavioral performance due to an enhanced pro-inflammatory environment in the CNS. New mouse tumor models represent a unique opportunity to test our hypothesis. Our project utilizes immunotherapy (antiCTLA4) preceding precision CT guided RT delivered via Small Animal Radiation Research Platform (SARRP) to test clinical trial treatment plans. BALB/c mice with or without CT26 colorectal carcinomas or C57BL/6 mice with or without 3LL lung carcinoma were treated with antiCTLA4 intraperitoneally (i.p.) on days 0, 3, and 6. Radiation was delivered using a CT1 beam on day 7 (A). Control groups included untreated mice without tumors (i.e., sham RT), CT26-bearing mice treated with antiCTLA4 or sham RT, and 3LL-bearing mice treated with antiCTLA4 or sham RT. All mice were assessed using a number of behaviors 7 days following treatment: (1) locomotor activity, (2) exploratory behavior, (3) memory, and (4) anxiety. Though treatment combinations were intended to increase microglial activation, no increases were seen. BALB/c mice treated with combined antiCTLA4 and 20Gy RT (A) demonstrated decreased locomotor activity, decreased exploratory behavior, and decreased anxiety compared to untreated mice (B). Microglial activation was not increased on day 7 in BALB/c tumor mice, but increased in C57BL/6 tumor mice. These findings may be considered in cancer patients receiving similar treatments. Currently, we are assessing immune and synapse-related molecular markers in the brains of these mice that may be associated with these behavioral changes. A more profound effect will be shared at the meeting.

Materials and Methods

Mice model. Two cohorts of 2-4 month-old female wild-type mice were used. Cohort 1 was a CT26 colorectal carcinoma model in BALB/c mice and Cohort 2 was a 3LL lung carcinoma model in C57BL/6 mice. Mice were obtained from the Jackson Laboratory, Bar Harbor, ME and tumors established and treated by Dr. Michael Gough of the Providence Cancer Center (Portland, OR). There were four experimental groups: 1) healthy mice, no treatment; 2) healthy mice, combined antiCTLA4 and irradiation; 3) tumor model, no treatment; 4) tumor model, combined treatment. Each group consisted of 10 mice. For the tumor models, 3LL or CT26 cells were injected subcutaneously into the right flank. 250µg AntiCTLA4 (Clone 410D, BioxCell, West Lebanon, NH) antibody was delivered intraperitoneally at day 7 prior to radiation therapy. 20Gy CT-guided radiation was delivered at day 7 using a Small Animal Radiation Research Platform (Xstrahl, Guelph Medical, Suwanee, GA).

Behavioral and Cognitive Testing. Behavioral and cognitive testing was performed following antiCTLA4 treatment and irradiation. All mice were tested using the following paradigms: 1) Rotarod for balance, grip strength, and motor coordination; 2) Open Field for anxiety, general locomotor activity, and willingness to explore; 3) Novel Object for recognition memory; 4) Water maze for hippocampal-dependent spatial learning and memory; and 5) Fear Conditioning for hippocampal-dependent contextual fear conditioning and hippocampus-independent cue fear conditioning.

Immunohistochemistry. Following behavioral testing, 4 mice from each experimental group were perfused for immunohistochemistry and confocal microscopy. Brain sections were processed for CD-68 immunoreactivity. CD-68 was used to identify reactive microglia.

Introduction

• Cognitive impairments are common but poorly understood complications of cancer and cancer therapy involving chemotherapy and/or radiation.
• The temporal lobe, and in particular the hippocampus, seems particularly sensitive to detrimental effects of chemotherapy and radiation on cognition³.
• A pro-inflammatory profile in the brain has been associated with behavioral changes and cognitive impairments, including memory impairments and decreased executive function⁴.
• Enhanced pre-inflammatory tumor environments are associated with increased survival and tumor regression, while anti-inflammatory tumor environments are correlated with severely limited survival⁵.
• An improved immune environment in the tumor at the time of treatment also increases the efficacy of radiation therapy (Figure 1).
• Though immunotherapy enhances the immune response of patients with poor tumor infiltrate profiles, it is unclear how immunotherapy might modulate the effects of radiation on the brain⁶.

Discussion

• Combination treatment of antiCTLA4 immunotherapy and radiation therapy decreases measures of anxiety in healthy mice and BALB/c tumor mice. However, the opposite pattern is seen in C57BL/6J tumor mice.
• In mice with tumors, combination treatment increases or decreases measures of anxiety depending on genetic background.
• Healthy mice treated with combination therapy and mice with tumors have increased activation of microglia.
• Mice with tumors treated with combination therapy have levels of microglial activation similar to healthy controls.
• Microglial activation does not appear to be associated with changes in anxiety.
• These results indicate the importance of including healthy mice (without tumors) and considering genetic background in assessing the effects of combination therapy on the brain.
• Additional immunohistochemistry and cytokine assays using tissues from these mice may further elucidate the mechanisms underlying these effects.

Results

Immune-mediated control of CT26 colorectal tumors in BALB/c mice RESULTS

Figure 1. Use of antiCTLA4 to inhibit the suppression of the anti-tumor T cell response increases responses to radiation therapy and improves survival outcomes, an effect that is T cell dependent. (A) Average tumor diameter in mice left untreated (NT) or treated (RT) with radiation alone or radiation combined with CD8 depletion on days 10 and 17. (B) Growth of individual CT26 tumors in mice (i) untreated, (ii) treated with 250µg antiCTLA4 on day 0, (iii) treated with 200Gy RT on day 14, (iv) treated with 250µg antiCTLA4 and 20Gy RT on day 7, and (v) treated with 200Gy RT on day 14.

CT-guided treatment of subcutaneous tumors using the SARRP

Figure 2. Radiation was delivered to treatment groups using the Small Animal Radiation Research Platform (SARRP). Using a cone-beam CT scan with 360 projections, the tumor was visualized using the SARRP and then the center of the tumor was treated with 20Gy using a 10mm×10mm collimator at a 50 degree angle to deliver dose to the incisor with minimal dose to radiosensitive organs.

Measurements of anxiety in BALB/c and C57BL/6J mice receiving combination therapy

Figure 3. A) BALB/c mice with or without CT26 tumors treated with combination therapy show decreased measures of anxiety as demonstrated by number of entries into the center in the open field (p=0.02). B) After combination treatment, C57BL/6J mice without 3LL tumors show decreased measures of anxiety, whereas those with 3LL tumors show increased measures of anxiety (p=0.03). n=10 female mice/group.

References

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