

THE ROLE OF CCL20 IN MEDIATING REGULATORY T CELL INFILTRATION AND RESISTANCE TO RADIOTHERAPY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background Radiotherapy (RT) is commonly used to treat solid tumors but its efficacy varies widely. In non-virally driven head and neck cancer (HNC), radioresistance is responsible for most cases of tumor progression and tumor recurrence. The combination of RT and immunotherapy (IT), in the form of anti-PD-1/PD-L1, improved survival for a minority of patients with tumors that have pre-existing immunity or which are susceptible to RT-induced tumor immunity. In contrast, tumors with an immunosuppressive microenvironment show resistance to RT and/or IT. Regulatory T cells (Tregs) have been shown to be prevalent in HNC tumors and can promote treatment resistance. We identified the chemokine, CCL20 as a factor that can promote infiltration of Tregs into HNC tumors. CCL20 is secreted by the cancer cell and binds to its sole receptor, CCR6, expressed on Tregs. We therefore hypothesized that radiation induces CCL20 secretion resulting in infiltration of Tregs and radioresistance. We further hypothesized that blocking CCL20 in HNCs where CCL20 is induced in response to RT can decrease tumor growth.

Methods Human and murine HNC cell lines (SCC9, Cal27, MOC1, MOC2) were irradiated at doses of 2Gy or 10Gy. Conditioned media (CM), RNA and protein were obtained 24h and 72h after radiation. The concentration of CCL20 was analyzed using a chemokine array. Gene expression was determined using qPCR. For in vivo studies, the MOC2 cell line was used. Mice were inoculated in the buccal cavity. Neutralizing CCL20 antibody was administered alone and in combination with RT. Blood samples were collected before and after RT for analysis of serum levels of CCL20.

Results Gene expression analysis showed that Cal27 and MOC2 tumors had a gene signature associated with immune-suppression and Treg cell infiltration. In contrast, SCC9 and MOC1 tumors displayed a gene signature associated with an inflamed microenvironment. In vitro, radiation induced a significant increase in CCL20 gene expression and secreted CCL20 in Cal27 and MOC2 cells relative to control. In contrast, MOC1 and SCC9 did not show a significant increase in CCL20 after radiation. In vivo, inhibition of CCL20 decreased tumor growth compared to control in MOC2 tumors. The combination of RT and anti-CCL20 showed a significant decrease in tumor growth compared to RT alone.

Conclusions Collectively, our data suggest that radiation promotes the induction of CCL20 in tumors with immune-suppressive mechanisms and inhibition of CCL20 can enhance the response to RT.

Ethics Approval All animal studies were conducted in accordance with the animal protection and ethics committee of the faculty of medicine at Université de Sherbrooke (Le Comité facultaire de protection des animaux). Protocol #: 2019-2333.

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