ASSESSING THE EFFECT OF POST-TRAUMATIC OSTEARTHRITIS ON MURINE TUMOR GROWTH AND RESPONSIVENESS TO IMMUNE CHECKPOINT BLOCKADE

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Background Immune checkpoint blockade (ICB) has revolutionized cancer treatment. The mechanisms of heterogeneous ICB responses (responder/non-responder), however, are poorly understood. Because of the increasing application of (neo)adjuvant ICB with tumor resection, the role of tissue injury caused by surgical procedures is a potential and usually overlooked factor in the generation of systemic anti-tumor immunity. Prior tissue injury has been shown to advance breast cancer progression.1,2 Whereas, the induction of senescent tumor cells and their senescence-associated secretory phenotype (SASP) within pancreatic cancer has been shown to remodel tumor vasculature, increasing ICB responsiveness.3 Thus, here we explore the role of cellular senescence induced by distant tissue damage on tumor growth. Anterior cruciate ligament (ACL) transection, a model of post-traumatic osteoarthritis, leads to senescent cell (SnC) accumulation in the joint and systemic SASP-mediated effects.4 We investigate the immunological and stromal changes arising with ACL injury and ICB within B16F10 murine melanoma, a classical non-responding model.

Methods ACL transection was performed prior to subcutaneous B16F10 inoculation. ICB treatment consisted of 4x intraperitoneal-injected 5mg/kg anti-PD1 monotherapy or anti-PD1/anti-CTLA4 combination therapy. Tumor growth was monitored via caliper measurements. Serum was collected after ICB treatment for cytokine array. Tumors were collected at termination for histological assessment. To elucidate changes in vascularization and senescence within tumors due to injury and/or treatment, we performed histological (H&E) and immunofluorescent staining of CD31 and p16, respectively. We quantified the change in tumor vessel number and size using QuPath and used HALO image analysis to quantify p16+ and CD31+ cells and their proximity.

Results ACL injury either 1- or 7-weeks prior to tumor injection significantly increased B16F10 responsiveness to ICB and survival. In tumors from ACL-injured mice treated with ICB, immunofluorescent staining showed significantly more p16+ SnCs and CD31+ cells, with a greater number of p16+ SnCs within 10µm of vasculature compared to controls. QuPath analysis of immunofluorescent-stained tumors from ACL-injured+ICB-treated mice uncovered a significantly higher frequency of large blood vessels (surface area >10000µm²) and H&E staining showed vessels had larger lumen. Cytokine array revealed increased serum-levels of pro-inflammatory cytokines and angiogenic factors in ACL-injured+ICB-treated mice.

Conclusions Our data demonstrate that ACL injury significantly increases ICB responsiveness in a classically non-responding tumor model. ACL injury ameliorates tumor vascularization and increases the frequency of p16+ SnCs surrounding vasculature within ICB-treated tumors. The mechanism causing this effect is being elucidated. This study aims to develop predictive understanding of ICB responsiveness and provide mechanisms to sensitize non-responders.