DOXYCYCLINE REGULATABLE β-CATENIN DEMONSTRATES INDUCIBLE IMMUNE EVASION IN A MELANOMA GEM MODEL

Alexandra Cabanov*, Thomas Gajewski, Stefani Spranger, Elen Torres-Mejia. University of Chicago, Chicago, IL, United States; Koch Institute, Cambridge, MA, United States

Background Lack of response to checkpoint blockade immunotherapy has been linked to a deficiency of immune cell infiltration within the tumor microenvironment (TME). One demonstrated mechanism sufficient for the non-T cell inflamed TME is tumor cell-intrinsic activation of the β-catenin signaling pathway. Using genetically engineered mouse models (GEMMs), tumors constitutively expressing active β-catenin lack a robust endogenous T cell infiltrate and fail to respond to immunotherapies. In support of these mouse studies, human melanoma metastases with increased active β-catenin signaling exhibit decreased numbers of tumor infiltrating Batf3-driven cDC1 and CD3+ T cells. However, whether temporal activation and inactivation of β-catenin within the same developing tumor would alter immune cell infiltration is not known.

Methods A model was created in which tamoxifen-regulated Cre-recombinase mediates BRAFV600E oncogene activation and PTEN tumor suppressor gene deletion as well as expression of a doxycycline regulatable reverse transactivator. Upon administration of doxycycline via the drinking water to these animals, a non-degradable form of nuclear β-catenin becomes expressed. Immunofluorescence assays were performed assessing the β-catenin expression status in the tumor cells as well as immune cell infiltration within the TME. Additionally, immunotherapy efficacy experiments were performed.

Results We observed that administration of doxycycline to these animals drove expression of an active form of nuclear β-catenin. Activation of nuclear β-catenin resulted in a 2-fold decrease in the overall CD3+ T cells infiltration into the TME. Moreover, this decrease in immune infiltration also resulted in loss of anti-PD-L1 + anti-CTLA-4 therapy efficacy. We next performed studies assessing the kinetics with which β-catenin levels diminish upon doxycycline removal. Switching animals to regular drinking water resulted in complete reduction of nuclear β-catenin levels seven days after doxycycline removal. Interestingly, once nuclear β-catenin levels diminished, we observed re-infiltration of the TME however, these tumors remained unresponsive to anti-PD-L1 + anti-CTLA-4 therapy.

Conclusions We describe a novel mouse model in which we induce autochthonous melanoma tumors in mice along with inducible expression of a non-degradable, nuclear β-catenin modulated by doxycycline in the drinking water. While removing doxycycline resulted in re-infiltration of CD3+ T cells within the TME, it did not result in restored anti-PD-L1 + anti-CTLA-4 efficacy. Single cell RNA-seq and spatial transcriptomics are being performed to determine if an additional immune regulatory event becomes established when β-catenin is on from the birth of the tumor that needs to be overcome.

Acknowledgements This work was supported by the Wissler Fellowship from the University of Chicago (SS) K99/R00 (NCI; SS), and R35CA210098 (TG).