178 TARGETING ER-MITOCHONDRIAL DYNAMICS TO IMPROVE RESPONSES TO IMMUNE CHECKPOINT BLOCKADE

Katherine Cook, Elizabeth Stirling, Jessica Mackert, Mitra Kooshki, Adam Wilson, Wei Zhang, Pierre Triozzi, David Soto-Pantoja*. *Wake Forest School of Medicine, Winston-Salem, NC, USA*

Background One of the top challenges in cancer immunotherapy is 'understanding the molecular and cellular drivers of primary vs. secondary immune escape to immune checkpoint blockade therapies.' Although durable responses indicating immunologic memory are reported, most patients do not respond with tumor regression. In addition, recurrences are observed after initial response, suggesting the emergence of acquired resistance. Therefore, novel therapeutics are needed to enhance ICB treatment to halt melanoma progression and increase patient survival rates. Oxidative stress and hypoxia in the tumor microenvironment resulting in endoplasmic reticulum (ER) stress signaling associated with cancer therapy resistance. PKR-like endoplasmic reticulum kinase actions in the tumor microenvironment can occur by activation of the Unfolded Response (UPR). However, PERK also has functions that are not linked to canonical activation of UPR signaling. PERK is involved in the maintenance of ER with mitochondria-associated membranes (MAMs). This is important in glucose sensing and could impair metabolic reprogramming during T cell activation if disrupted.

Methods To determine ER-stress markers of patient response, we analyzed Peripheral Blood Mononuclear Cells by single-cell RNA sequencing (scRNAseq), 3D electron, and confocal microscopy to determine mitochondria/ER interactions. Antigen-mediated CD8+ T cell toxicity assay was performed using Pmel-1 transgenic mice. LC-MS Metabolomics and Seahorse mitochondrial respiration assays were used to determine CD8+ cellular energetics treated with anti-PD-1 in the presence or absence of PERK blockade. An in vivo murine tumor model (B16) was developed to determine whether PERK blockade sensitizes tumors to anti-PD1 therapy.

Results UMAP clustering of our scRNAseq data indicates a ~3-fold increase in PERK expression after the first treatment cycle in patients who did not respond to anti-PD-1 therapy ($n=12$, $^*p<0.05$). This was confirmed by plasma ELISA assay in a different cohort of patients ($N=50$, $^*p<0.001$). Furthermore, the expression of PERK in non-responder patients was associated with increased expression of TOX1 in the T-cell population, and induction of ER stress caused a transcriptional increase in TOX1 in T cells suggesting that ER stress may be associated with T cell dysfunction. Induction of ER stress limited antigen-mediated CD8+ T cell killing; however, blockade of PERK enhanced the cytolytic capacity of CD8+ T cells over 4-fold, clearing tumor cells even under ER stress-induced circumstances ($n=4$, $^*p<0.01$). Examination of ER-mitochondria interactions by confocal and 3D electron microscopy suggested increased co-localization of these two organelles in lymphocytes of responder patients to anti-PD1 therapy. Blockade of PERK in combination with anti-PD1 treatment resulted in a 64% reduction in tumor volume compared to control and an over 40% reduction compared to anti-PD1 monotherapy ($n=7$, $^*p<0.002$). This suggests that blockade of PERK enhances αPD-1 efficacy. PCA from metabolomics of tumor-infiltrating T cells suggested a differential metabolic signature that separates the PD1 treated group from control, PERK blockade, and combination-treated animals. Furthermore, blockade of PERK increased glycolytic flux and mitochondrial respiration of CD8+ T cells. Thus, blockade of PERK supports metabolic reprogramming to support T cell effector function under ER stress conditions.

Conclusions Our data show that PERK expression may serve as a potential clinical marker of ICB response. Our preliminary evidence indicates that Targeting PERK improved the efficacy of anti-PD1 therapy by modulating metabolic signaling of CD8+ T cells. Thus, targeting PERK in the tumor microenvironment may inhibit pro-oncogenic and immunosuppressive signaling from enhancing ICB responsiveness.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0178