THE ROLE OF CHOLESTEROL HANDLING IN GAMMA DELTA T CELL CYTOTOXICITY AGAINST HIGHLY IMMUNE SUPPRESSIVE PANCREAS DUCTAL ADENOCARCINOMA

Johnathan Ebben*, 1David Turcik, 2Md Shahadat Hassan, 1Austin Stram, 1Ethan Lin, 2Nicholas J Hess, 2Zachary Mayhew, 3Melissa Kinney, 2Christian Capitini, 2Jeremy Kratz.
1University of Wisconsin Carbone Cancer Center, Madison, WI, USA; 2Nicholas J Hess, 2Zachary Mayhew, 3Melissa Kinney, 2Christian Capitini, 2Jeremy Kratz.
1University of Wisconsin Carbone Cancer Center, Madison, WI, USA; 2University of Wisconsin-Madison, Madison, WI, USA; 3University of Wisconsin-Madison College of Engineering, Madison, WI, USA

Background Pancreas ductal adenocarcinoma (PDAC) is highly resistant to most therapies, including immunotherapy. PDAC create highly immunosuppressive tumor microenvironments, yet also contain infiltrates of exhausted gamma delta T cells (gDTs). We sought to determine how PDAC suppresses gDT activation within the tumor-immune microenvironment through production of secreted factors. Using RNAseq we identify dysregulated cholesterol metabolism as a potential mechanism induced by PDAC in gDT which may impair gDT anti-tumor cytotoxicity.

Methods PDAC organoids were developed from patient biopsies. Media in which PDAC organoids were cultured were harvested (conditioned media), modelling secreted factors produced by PDAC. Circulating gDTs were isolated from the peripheral blood of healthy allogeneic donors using negative selection (StemCell Tech) after gradient centrifugation, and expanded with IL-2, IL-15, and zolecdronic acid. Healthy gDTs were then exposed to PDAC-conditioned media for 72 hours to simulate the in vivo PDAC secretome and incubated with PDAC organoids at an effector:target ratio of 5:1. After 48 hours, a fluorescent cleaved caspase 3/7 dye was added. Live cell imaging was performed (BioTek) to assess organoid area stained positive for cleaved caspase 3/7.

Results Culture of healthy donor gDTs with PDAC secretome is associated with decreased gDT killing of PDAC organoids across 2 separate patient PDAC organoid lines. Greater than 50% reduction in total cleaved caspase 3/7 area of organoids was seen with PDAC-conditioned gDTs versus gDTs in base media (figure 1A; each data point represents a separate measured organoid). Bulk RNAseq of gDTs (n = 3 biological replicates of 3 separate conditioned media exposed and control media exposed gDTs) following conditioning with PDAC secreted factors results in increased cholesterol efflux (ABCG1 upregulated by 16 fold vs control, p<0.0001); decreased cholesterol uptake (LDLR downregulated 4 fold vs control, p<0.0001), and downregulation of cholesterol biosynthesis in gDTs (HMGCR downregulated 2.7 fold vs control, p<0.0001) (figure 1B). Preliminarily, addition of 10ug/mL of low-density lipoprotein (LDL) to gDTs exposed to PDAC conditioned media increased overall gDT mediated PDAC killing (n = 236 organoids selected of 3 separate conditioned media, figure 1C).

Conclusions gDTs are functionally suppressed by PDAC secretome, potentially driven by decreased uptake, increased efflux, and decreased biosynthesis of cholesterol in gDTs (figure 1D). In a preliminary experiment, supplementation of the tumor microenvironment with LDL appears to restore gDT cytotoxicity against PDAC. Modulation of cholesterol handling in the tumor-immune microenvironment may enhance immune cell effector therapy for patients with PDAC.

Acknowledgements This work was supported by a NCI/NIH T32 CA009135, as well as a University of Wisconsin Trainee Pilot Award (Department of Medicine; JDE), University of Wisconsin Carbone Cancer Center (UWCCC) pilot grant (Project AAK9374 from the UWCCC Pancreatic Cancer Task Force; JDE, JDK, CMC), St. Baldrick's Empowering Pediatric Immunotherapies for Childhood Cancer Team Grant, and the MACC Fund (C.M.C). We would like to thank the UWCCC core facilities, who are supported in part through NCI/NIH P30 CA014520.

Ethics Approval Organoids used in the course of this work were developed under an IRB approved by the University of Wisconsin-Madison (Protocol B00000976, PI Dr. Jeremy Kratz, MD), with all patient tissue collected and used with informed consent.

Abstract 1471 Figure 1 (A,C) gDT were pre-conditioned with either organoid growth media or media conditioned by organoids from Patient A or B for 72 hours. gDT were then exposed to PDAC organoids for 48 hours: caspase 3/7 activity as a% of the total area of the measured organoids was determined by live cell imaging. Each dot represents one separate organoid. (B) Schematic of key RNAseq results superimposed on cholesterol handling pathways. (D) Graphical schematic of potential impact of PDAC factors on key elements of cholesterol handling that may impact gDT anti-tumor cytotoxicity.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1471