187 MULTI-OMIC SINGLE-CELL PROFILING DEMONSTRATES THAT COMPETITION FOR FATTY ACIDS AND FATTY ACID OXIDATION ENABLES TUMOR-INFILTRATING LYMPHOCYTE FUNCTION AND SURVIVAL

[1]Melisa Angela Paniagua*, [2]Cara Haymaker, [1]Jay R Adolacion, [1]Xingyue An, [2]Caitlin Creasy, [1]Mohsen Fathi, [1]Ali Rezvan, [3]Tamar Geiger, [3]Michal Harel, [4]Jonathan Robinson, [1]Amit Amritkar, [2]Scott Woodman, [2]Patrick Hwu, [2]Chantale Bernatchez, [1]Navin Varadarajan. *[1]University of Houston, Houston, TX, USA; [2]UT MD Anderson Cancer Center, Houston, TX, USA; [3]Tel Aviv University, Israel, Israel; [4]Chalmers University of Technology, Sweden, Sweden*

Background Adoptive transfer of ex vivo expanded tumor-infiltrating lymphocytes (TIL) have shown durable responses in metastatic melanoma, yet these responses are unpredictable. Bioenergetics dictates the function and fate of adoptively transferred human T cells within the tumor microenvironment but the nature of metabolic competition leading to T-cell function and dysfunction are incompletely understood.

Methods We integrated the profiling of TIL co-cultured with their autologous primary tumor cells with the aid of a suite of high-throughput single-cell functional assays, transcriptional, and proteomic assays. We validated the results of the model using flow cytometry and confocal microscopy. Association of functional features with clinical outcome was assessed.

Results Timelapse Imaging Microscopy In Nanowell Grids (TIMING) demonstrated that while TIL frequencies in killing autologous tumor cells are equivalent across the donors, R-TIL had a significantly higher survival rate than NR-TILs. Tumor cells from NR patients had higher motility and showed increased elongation compared to R-tumors. RNA-sequencing (RNA-seq) and proteomics showed that NR-tumors were enriched in pathways associated with utilization of fatty acids (FAs) and adipogenesis, as well as cancer cell metastasis, cellular motility, adhesion, and migration. Candidate genes associated with ameboidal migration (MYH9, MYH2; WNT5B and SERPINE1) and FA utilization (CD36 and PPARG) were enriched in the NR-tumors. Flow cytometry and confocal microscopy confirmed that NR-tumors showed increased CD36 expression and FA uptake compared to R-tumors. To simulate metabolic competition, we co-cultured the TIL with autologous tumors and sorted TIL for RNAseq. The R-TIL were enriched in pathways related to mitochondrial and carbohydrate metabolism; fatty acid oxidation (FAO), and long-chain FAs with a direct enrichment in fatty acyl CoA biosynthesis and both peroxisomal and mitochondrial FAO. Since patient-derived TILs were limiting for metabolomics type assays, we utilized genome-scale metabolic models to infer relevant metabolic pathways by comparison to the human metabolic Atlas (HMR2). At the level of individual metabolites, the significantly enriched metabolites within R TILs were dominated by peroxisome and mitochondria derived fatty acyl-CoA: e.g. palmitoyl-CoA, linoleoyl-CoA, and oleoyl-CoA. We utilized flow cytometry and confocal microscopy to perform pulse-chase assays with FAs for validation. R-TILs showed an increased accumulation of FA into the mitochondria confirming a direct role for TIL FAO.

Conclusions Efficient competition for FAs is a key attribute of T-cell efficacy in ACT. R-TILs are able to utilize FAs via FAO when in competition with autologous tumor cells whereas NR tumors effectively uptake and store FAs preventing T-cell function.

Acknowledgements This abstract was supported by the NIH (R01CA174385); CPRIT (RP180466); MRA Established Investigator Award to NV (509800), Welch Foundation (E1774); NSF (1705464); CDMRP (CA160591); Owens Foundation (NV). We would like to acknowledge the MDACC Flow Cytometry and Cellular Imaging Core facility for the FACS sorting (NCI P30CA16672), UH Seq-n-edit core for RNA-seq, Intel for the loan of computing cluster, and the UH Center for Advanced Computing and Data Systems (CACDS) for high-performance computing facilities.

Trial Registration protocol (2004–0069)

Ethics Approval Approved by the Institutional Review Board (IRB) of the MD Anderson Cancer Center (Houston, TX) and an FDA- approved Investigational New Drug (IND) application (NCT00338377)

http://dx.doi.org/10.1136/jitc-2021-SITC2021.187