## 304 DISCOVERING T CELL PROTEOME TURNOVER DYNAMICS TO ENHANCE PERSISTENCE IN SOLID TUMORS

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**Background** Adoptive T cell therapies are widely considered to be the next frontier in cancer immunotherapy treatment but are currently only effective at treating cancers that do not form solid tumors. Tumor-infiltrating lymphocytes (TILs) become 'exhausted' in the hostile tumor environment where they lose effector function and express numerous inhibitor receptors, hindering their ability to control tumor progression. TILs face a host of environmental stresses as they encounter the tumor microenvironment including lack of glucose, hypoxia, acidosis, and ROS, all of which contribute to T cell dysfunction. We seek to understand the proteins T cells depend on to ensure adequate plasticity when adapting to environmental stress. To do this, we have employed proteomic approaches exploring global proteome turnover changes and the differential expression of E3 ubiquitin ligases associated with T-cell activation and exhaustion. E3 ubiquitin ligases are the master regulators of proteome turnover and have the potential to drastically alter proteome renewal and stability.

**Methods** For T-cell co-stimulation, Human primary CD8+ T-cells were isolated from three donors and stimulated with either αCD3/αCD28 or αCD3/α41BB. Cells were harvested on day 4 for proteomic analysis to look for differential E3 ligase expression. For T-cell exhaustion, CD8+ T-cells were stimulated using αCD3/αCD28 either on Day 0 (acute stimulation), or every 2 days for 8 days (chronic stimulation). Cells were harvested at day 8 for proteomic analysis to look for differential E3 ligase expression and to assess global proteome turnover changes (>4000 half-lives). E3 ligases lost during T cell exhaustion were over expressed in mouse (OT-1) tumor specific T-cells and subjected to *in vitro* T-cell killing experiments.

**Results** As expected, CD28 co-stimulation led to a glycolytic dependency while 41BB co-stimulation led to a dependency on OxPhos. T-cell exhaustion revealed an increase in a glycolytic phenotype, elevation of exhaustion transcription factors (e.g., TOX2, NR4A) and inhibitory receptors (e.g., PD1, CTLA4). Proteomic analysis identified 30 differentially expressed E3 ubiquitin ligases associated T-cell co-stimulation (CD28, 41BB) and 37 associated with T-cell exhaustion with an adjusted p value < 0.05. Data will be shown for global proteome turnover changes in exhausted T cells.

**Conclusions** Our overarching hypothesis is that the ability of a T-cell to persist and function in the hostile tumor microenvironment can be enhanced by dynamic control of its proteome through advantageous E3 ubiquitin ligases. Our work highlights the contribution of E3 ubiquitin ligases to T-cell biology and necessitates their further investigation.

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