Proteome turnover dynamics profiling reveals destabilization of key metabolic machinery during T cell exhaustion

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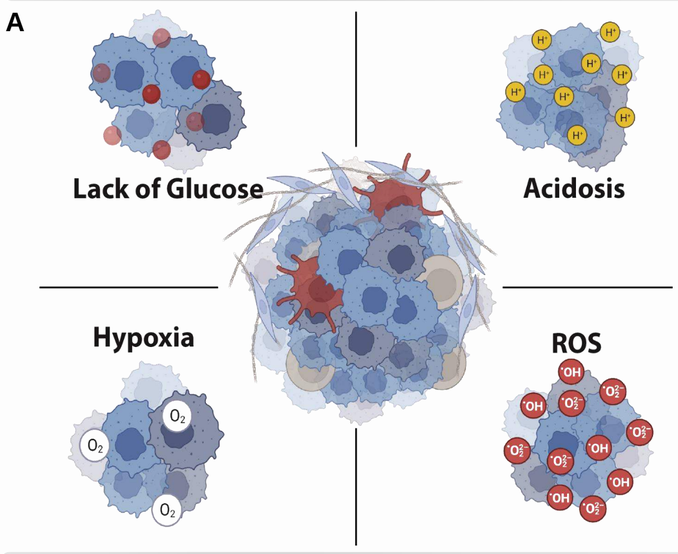
Abstract

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 (>6000 protein half-lives) and the differential expression of E3 ubiquitin ligases (20 E3 ligases quantified) associated with T-cell activation and exhaustion. In our model of T cell exhaustion, we found destabilization of the T Cell Receptor (TCR) complex leads to a loss in expression. Surprisingly, many glycolytic enzymes are destabilized without a loss in total abundance, indicating a rapid renewal and possible compensation for protein damage and misfolding. 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.

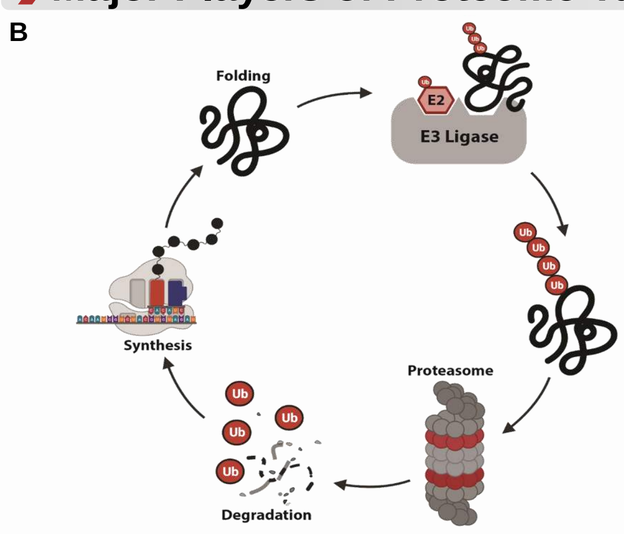
Introduction

Environmental Stresses of the Tumor Microenvironment



1A. Once a T cell finds its way into the tumor, it must function and persist within the hostile solid tumor environment. TILs face a host of environmental stresses as they encounter the microenvironment of the tumor including lack of glucose, acidosis, hypoxia, and reactive oxygen species (ROS) - all of which TILs must overcome. TILs become “exhausted” and are unable to survive the harsh environment. They lose all effector function and express many inhibitory receptors like CTLA4 and PD-L1 which are known to exert repressive function through inhibition of co-stimulatory signaling.

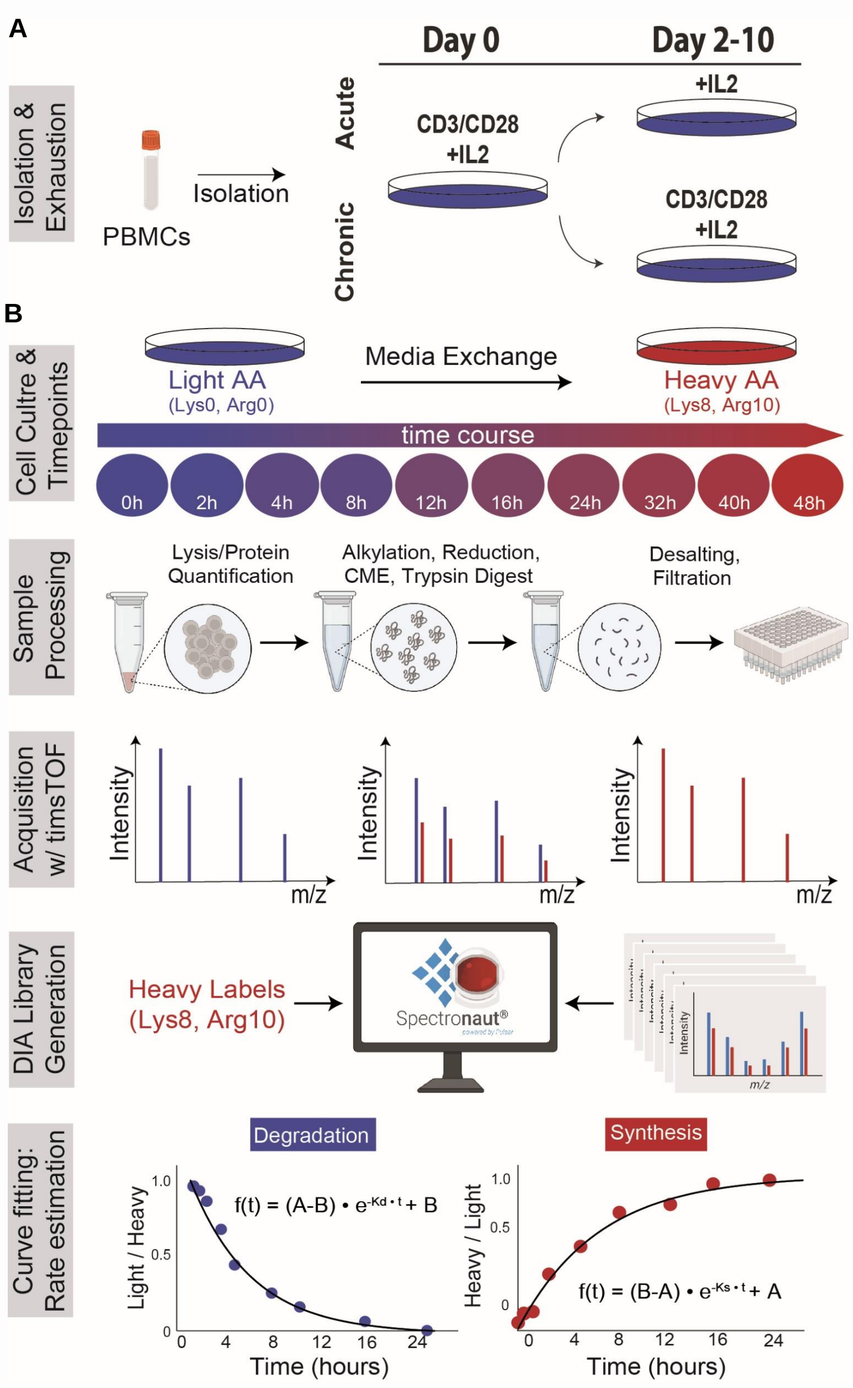
Major Players of Proteome Turnover



1B. The ability of a cell to dynamically adjust proteome composition is essential during stress. For that reason, protein turnover rates are optimized to 1) balance energy-saving stability and 2) to rapidly activate or inhibit specific signaling pathways. E3 ligases are the master regulators of proteome turnover and have the potential to drastically alter proteome renewal and stability. E3 ligases are enzymes that transfer ubiquitin to specific proteins, targeting them for degradation via the proteosome. We propose that manipulation of E3 ligases in CAR T-cells will enhance their ability to circumvent the detrimental environmental stresses of the tumor microenvironment.

Methodology

Protein Turnover Proteomic Method

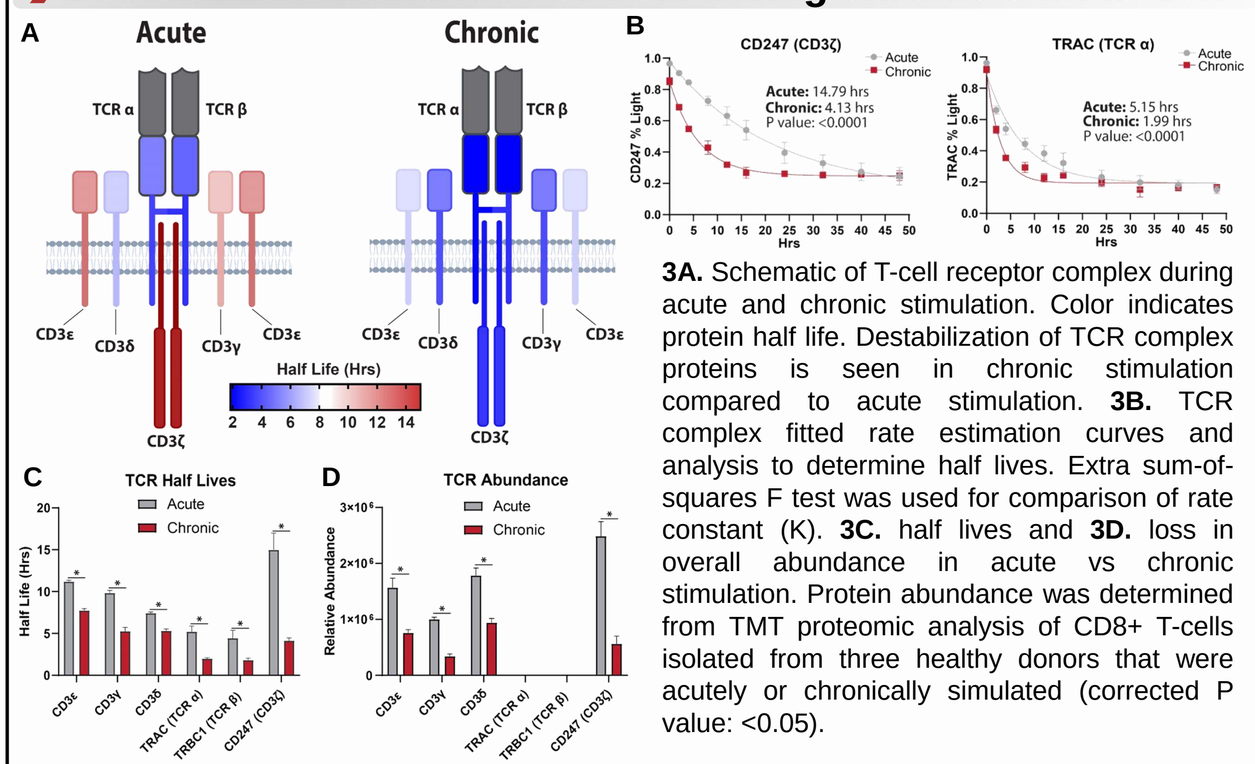


2A. Naïve CD8+ T-cells were isolated from PBMCs, activated using anti-CD3/CD28 mAb, and cultured in media containing light amino acids. At day 2, chronic T-cells were stimulated every 2 days with CD3/CD28 + IL-2 while acute T-cells received IL-2 only.

2B. On day 10, cells were switched to media containing heavy amino acids and harvested at various time points (0-48 hours). 30 µg of protein was trypsin digested using a Chloroform Methanol Extraction procedure. For Data independent Analysis mass spectrometry analysis, tryptic peptides are resolved by in-line acidic pH reverse-phase chromatography with an EvoSep One UPLC system coupled to a Bruker timsTOF Pro. High-resolution MS and MS/MS data was collected, and proteins identified using a Spectronaut custom library. This workflow generated >7,000 proteins IDs per sample. Time points were analyzed separately where percent heavy and light peptides were quantified and normalized to a rate estimation curve. Peptides with an R^2 >.9 were averaged for final rate estimation. Data represents two independent experiments (n=2) from a single healthy donor. This approach yielded ~6000 protein half life measurements present in all samples.

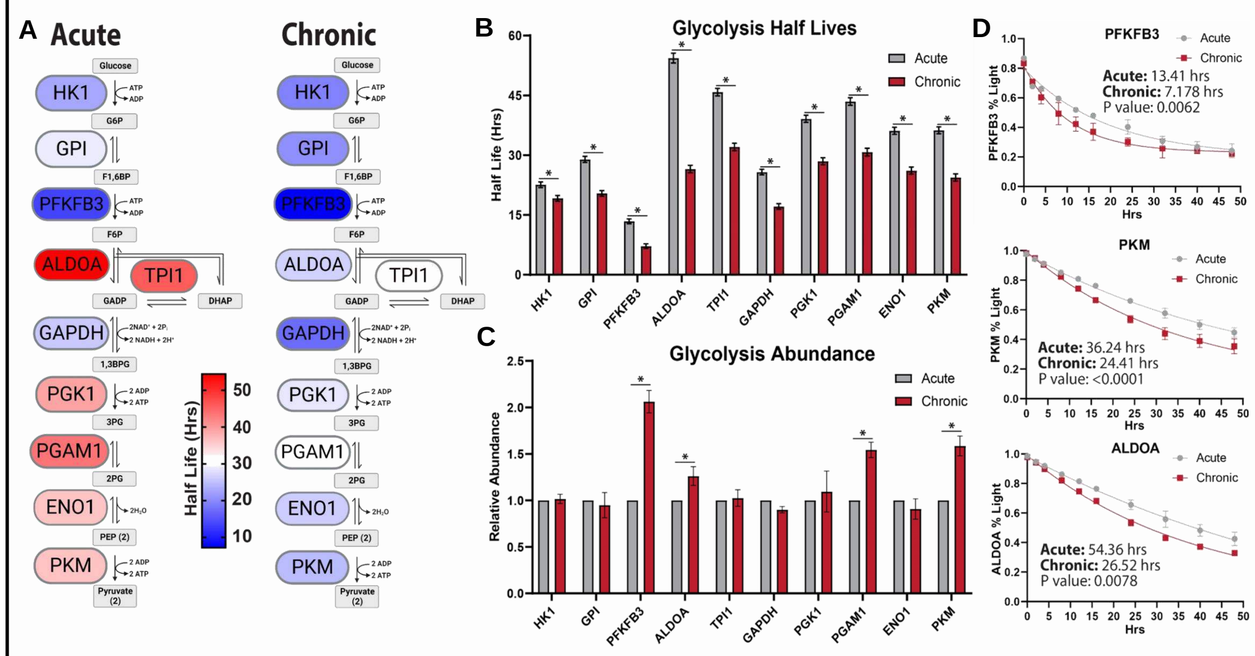
Results

Destabilization and loss of TCR during chronic stimulation



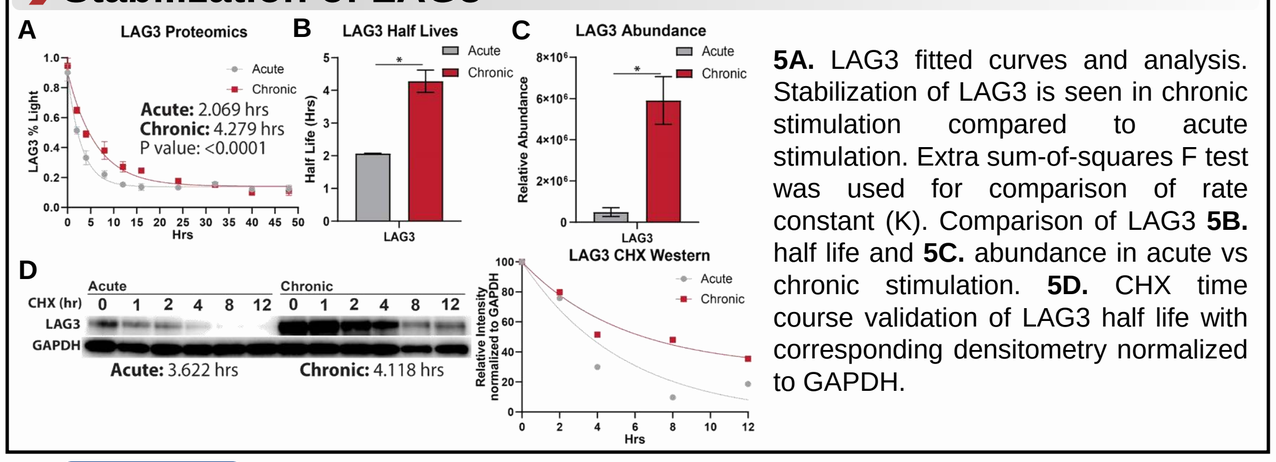
3A. Schematic of T-cell receptor complex during acute and chronic stimulation. Color indicates protein half life. Destabilization of TCR complex proteins is seen in chronic stimulation compared to acute stimulation. **3B.** TCR complex fitted rate estimation curves and analysis to determine half lives. Extra sum-of-squares F test was used for comparison of rate constant (K). **3C.** half lives and **3D.** loss in overall abundance in acute vs chronic stimulation. Protein abundance was determined from TMT proteomic analysis of CD8+ T-cells isolated from three healthy donors that were acutely or chronically simulated (corrected P value: <0.05).

E3 Ligases associated with co-stimulation



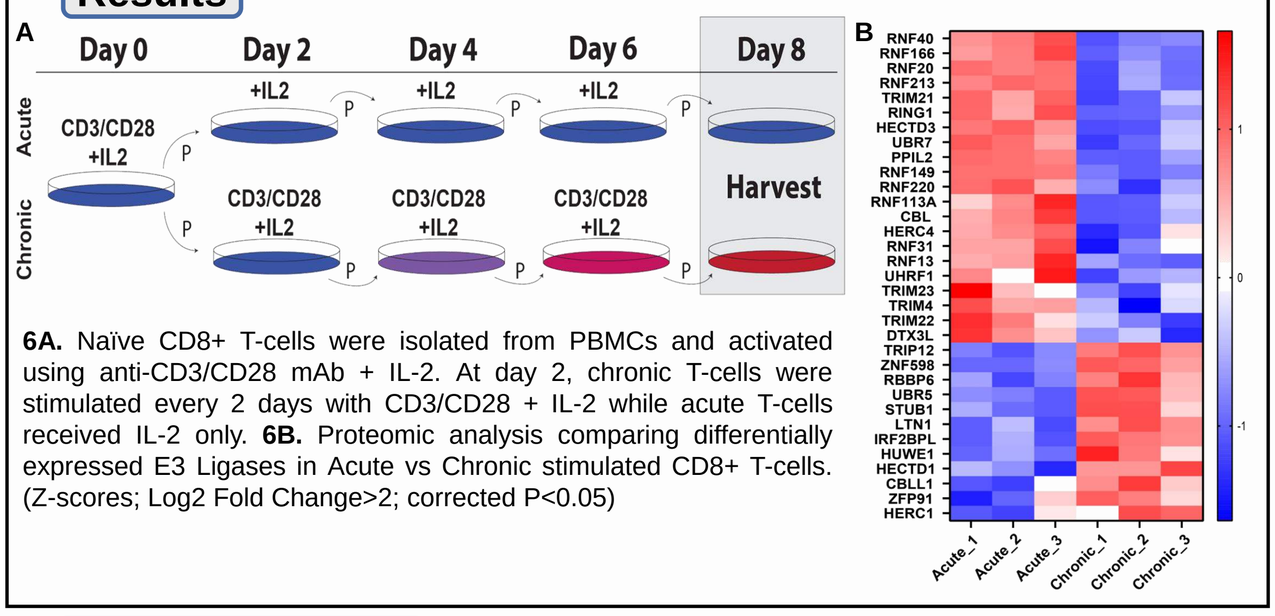
4A. Schematic of enzymes found in metabolic pathway of glycolysis during acute and chronic stimulation. Color indicates protein half life. Rapid renewal of glycolytic enzymes is seen in chronic stimulation (mimicking T-cell exhaustion) compared to acute stimulation. Comparison of glycolytic enzyme **4B.** half lives and **4C.** gain in overall abundance in acute vs chronic stimulation. Protein abundance was determined from TMT proteomic analysis of CD8+ T-cells isolated from three healthy donors that were acutely or chronically simulated (corrected P value: <0.05). **4D.** Glycolytic enzyme fitted rate estimation curves and analysis to determine half lives. Extra sum-of-squares F test was used for comparison of rate constant (K).

Stabilization of LAG3



5A. LAG3 fitted curves and analysis. Stabilization of LAG3 is seen in chronic stimulation compared to acute stimulation. Extra sum-of-squares F test was used for comparison of rate constant (K). Comparison of LAG3 **5B.** half life and **5C.** abundance in acute vs chronic stimulation. **5D.** CHX time course validation of LAG3 half life with corresponding densitometry normalized to GAPDH.

Results



6A. Naïve CD8+ T-cells were isolated from PBMCs and activated using anti-CD3/CD28 mAb + IL-2. At day 2, chronic T-cells were stimulated every 2 days with CD3/CD28 + IL-2 while acute T-cells received IL-2 only. **6B.** Proteomic analysis comparing differentially expressed E3 Ligases in Acute vs Chronic stimulated CD8+ T-cells. (Z-scores; Log2 Fold Change>2; corrected P<0.05)

Acknowledgements

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