

PROTEASOME MEDIATED PROTEIN CATABOLISM FUELS ANTITUMOR IMMUNITY

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Background The solid tumor microenvironment (TME) exposes CD8 T cells to metabolic stressors including nutrient and oxygen deprivation coupled with persistent antigen stimulation which work in concert to inhibit cell function.¹⁻⁵ Importantly, successful immunotherapy is predicated on CD8 T cells overcoming these hurdles to maintain proliferation and protein secretion. While recent advances in single cell RNA and ATAC sequencing have allowed for identification of genetic and epigenetic traits associated with enhanced antitumor T cell function⁶⁻⁷, little is known about the mechanisms responsible for controlling the translation of these instructions into effector functions. In this study, we sought to identify the mechanisms responsible for allowing sustained protein translation in the TME.

Methods Protein synthesis was monitored using a flow cytometry-based approach whereby the fluorescent analogue of methionine, L-homopropargylglycine (L-HPG), is incorporated into new forming polypeptide chains and quantified by flow cytometry through Click-IT chemistry.⁸⁻¹¹ Optimal antitumor T cells were generated via cytokine conditioning with IL-15 and then subjected to a series of *in vitro* tumor-T cell coculture systems and *in vivo* tumor growth experiments. Proteasome activity was monitored using a fluorescent activity probe via flow cytometry and pharmacologic intervention using the proteasome inhibitor MG-132 paired with metabolomics. Proteasome stimulator Cyclosporine A was used to validate our findings.

Results Using both human and mouse tumors we found that protein translation is repressed in T cells by the solid TME through activation of the unfolded protein response element p-eIF2a due to competition for glucose. Reprogramming T cells away from glucose dependency alleviated p-eIF2a mediated translation attenuation allowing for sustained translation under TME stress. Using metabolic and pharmacological approaches, we discovered that optimal antitumor T cells mitigate p-eIF2a through enhanced proteasome activity, protecting from translation attenuation enabling sustained cytokine synthesis in solid tumors that resulted in enriched tumor control. Additionally, we found the ability to access protein degradation via the proteasome complex was associated with metabolic programs previously linked to optimal antitumor immunity such as glutathione metabolism and gluconeogenesis.¹²

Conclusions These findings suggest that stress mediated attenuation of translation represents a cellular checkpoint which must be overcome for optimal tumor immunity. Our findings demonstrate protein degradation is a critical component of T cell tumor control and strategies that relieve the misfolded protein burden could be avenues to supplement current immunotherapy approaches.

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Ethics Approval Patients undergoing surgical removal of tumors granted consent via MUSC Biorepository surgical consent forms. This work was determined by MUSC Institutional Review Board to be exempt under protocol Pro00050181. Tissue samples were de-identified. Studies were conducted in accordance with the Declaration of Helsinki, International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS), Belmont Report, or U.S. Common Rule. All animal experiments were approved by both the Medical University of South Carolina (MUSC) Institutional Animal Care and Use Committee and the University of North Carolina at Chapel Hill (UNC) Division of Comparative Medicine. Mice were maintained by the Division of Laboratory Animal Resources at MUSC and Division of Comparative Medicine at UNC.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.1044>