CD8 T Cell Immunosurveillance is Suppressed by Lipid Signaling

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Summary

- To date, the mechanism(s) of how LPA affects CD8 T cell immunosurveillance and phenotype during tumor progression remain unknown.
- We propose LPA may be a potential T cell directed therapy **to improve efficacy of antigen-specific killing and metabolic fitness of CD8 T** cells which is applicable across solid tumor malignancies.

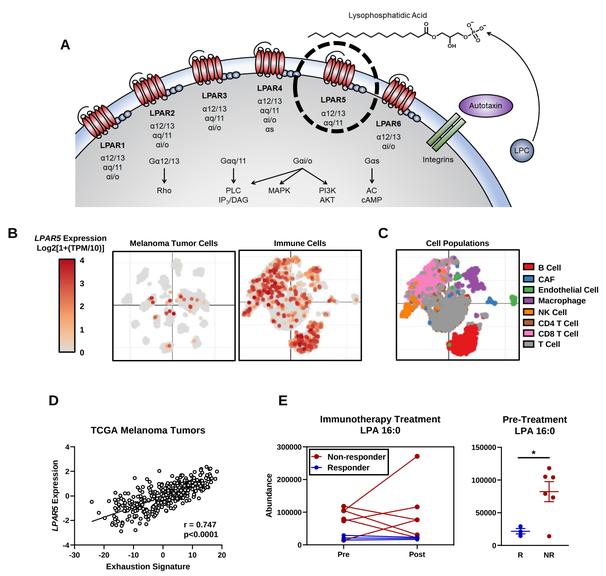

Figure 1. Lysophosphatidic acid is a prognostic marker in solid tumor malignancies. (A) Analysis of data from The Cancer Genome Atlas (TCGA) on progression free survival. (B) Single RNA sequencing tSNE plots of *LPAR5* expression in melanoma and immune cells. (C) tSNE plot of corresponding immune cell populations to panel C. (D) Spearman correlation analysis of *LPAR5* expression and exhaustion signature from bulk RNA sequencing on TCGA melanoma tumors. (E) Relative abundance of LPA in stage IV melanoma responder patients or non-responders.

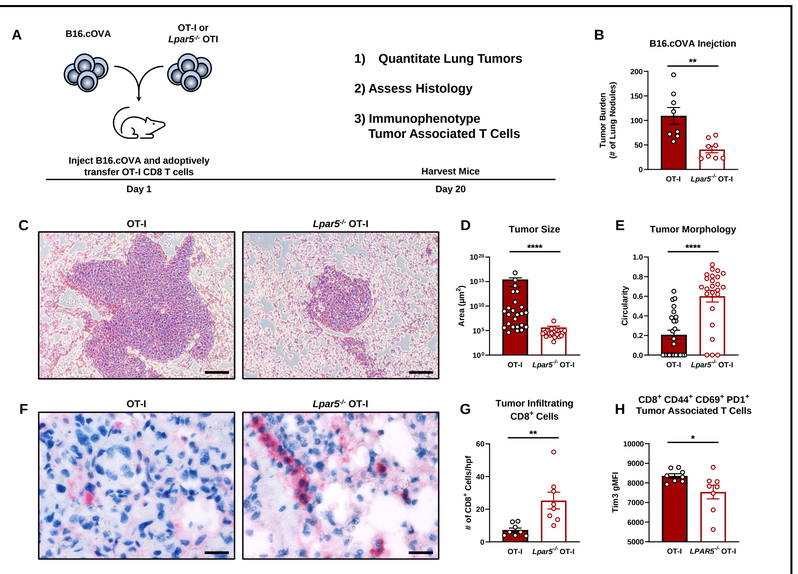

Figure 2. *Lpar5*$^{-/-}$ OT-I mice have improved tumor clearance and anti-tumor immunity. (A) Schematic showing study design. (B) Quantified tumor burden in the lung after intravascular injection of B16.cOVA cells. (C) Representative hematoxylin & eosin (H&E) histology images of the B16.cOVA tumor seeded in the lungs. Scale bars represent 100 μm. (D) Tumor area (μm^2) and (E) circularity quantified from H&E histology of B16.cOVA lung tumors. (F) Representative images of immunohistochemistry for CD8 on lung sections. (G) Quantification of intratumoral CD8 positive cells per high powered field (hpf, 400x). (H) Flow cytometric quantification of Tim3 expression.

Hypothesis: LPAR signaling promotes tolerogenic states through metabolic reprogramming and potentiates exhaustive differentiation to modulate anti-tumor immunity.

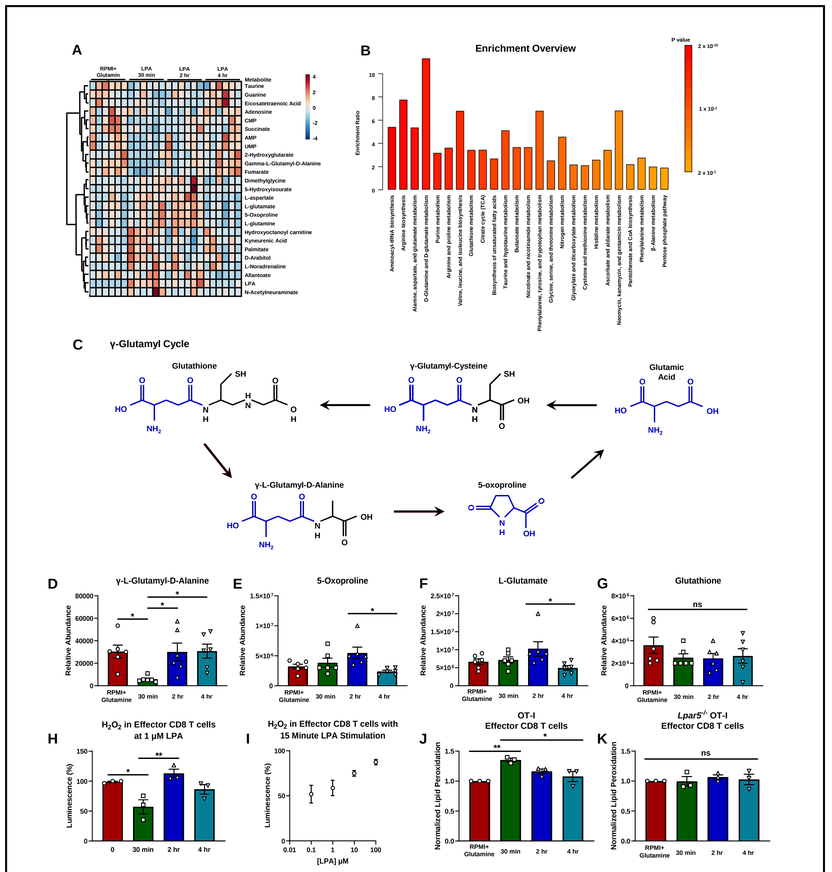

Figure 3. Lysophosphatidic acid rewires CD8 T cell metabolism and modulates reactive oxygen species. (A) Metabolic mass spectrometry on effector CD8 T cells. (B) Metabolite set enrichment analysis (MSEA) performed on raw data with KEGG analysis to determine enriched metabolic pathways. (C) Metabolic pathway of γ-glutamyl cycle. (D,E,F,G) Relative intracellular abundances of (E) γ-L-glutamyl-D-alanine, (E) 5-oxoproline, (F) L-glutamate, and (G) glutathione. (H,I) Direct measurements of H_2O_2 in effector CD8 T cells after LPA treatment. (J,K) Normalized lipid peroxidation as measured by TBARs in effector CD8 T cells after LPA treatment.

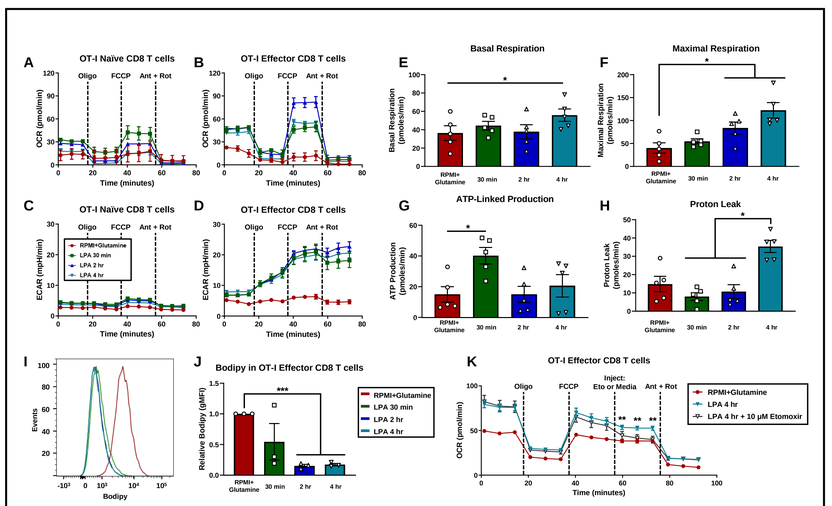

Figure 4. Lysophosphatidic acid shifts metabolism to consume fatty acids for oxidation. (A,B,C,D) Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) by both naïve and effector CD8 T cells given media without LPA (RPMI+Glutamine) or treated with 1 μM LPA. (E,F,G,H) Capacity calculations from Seahorse metabolic flux assay. (I,J) Flow cytometric analysis of bodipy in effector CD8 T cells. (K) Seahorse metabolic flux analysis performed with acute injection of etomoxir.

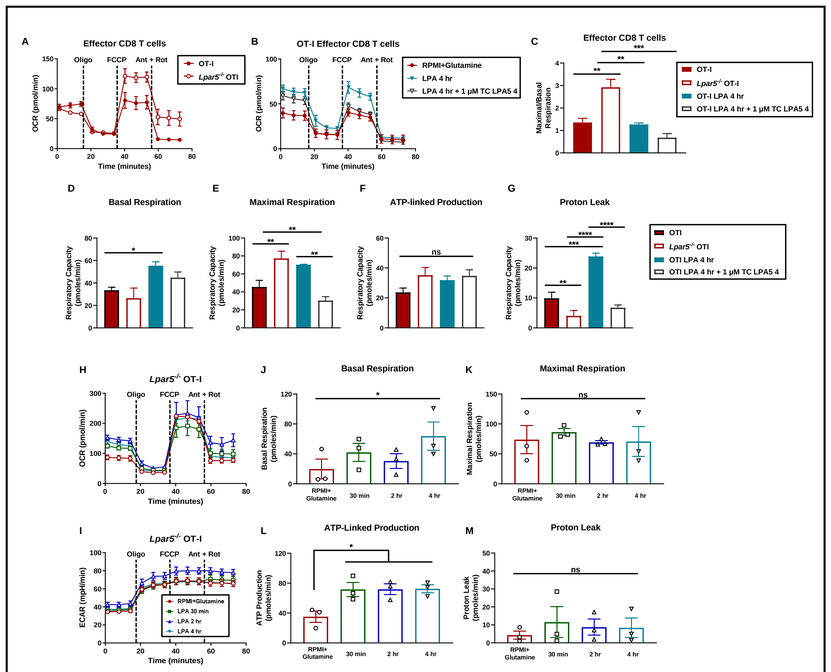

Figure 5. Lysophosphatidic acid receptor 5 modulates changes in maximal respiration and proton leak on effector CD8 T cells. (A, B) Seahorse metabolic flux assay performed on effector CD8 T cells from (A) an OT-I mouse or *Lpar5*$^{-/-}$ OT-I mice and (B) effector CD8 T cells treated with LPA receptor antagonist (TC LPA5 4). (C) Ratio of maximal respiratory capacity / basal respiratory capacity. (D,E,F,G) Capacity calculations from Seahorse metabolic flux assay. (H,I) Seahorse metabolic flux assay on effector CD8 T cells from an *Lpar5*$^{-/-}$ OT-I mouse cells treated with LPA. (J,K,L,M) Capacity calculations from Seahorse metabolic flux assay.

Future Directions

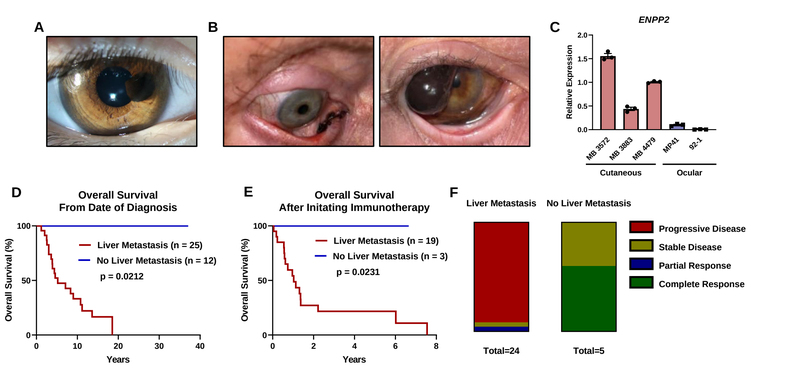

Figure 6. Lysophosphatidic acid signaling may be implicated in liver metastasis in ocular melanoma. (A,B) Ocular melanoma subtypes showing (A) uveal and (B) conjunctival melanomas. (C) *ENPP2* expression in ocular and cutaneous melanomas. (D,E) Overall survival ocular melanoma patients from (D) date of diagnosis and (E) after initiating immunotherapy. (F) Overall response rates of ocular melanoma patients to immunotherapy.

Conclusions

- LPA serves as a **lipid-regulated immune checkpoint** by modulating metabolic efficiency through LPAR5 signaling on CD8 T cells.
- Lipid signaling could be **exploited as a novel approach to prevent CD8 T cell dysfunction** and/or reinvigorate dysfunctional cells through modulating metabolic pathways to restore effector CD8 T cell function.

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