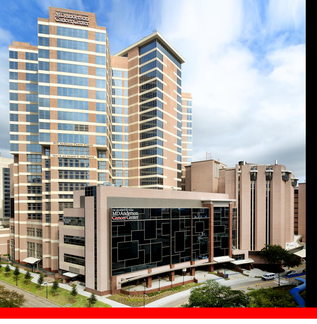

Exploring anti-PD-1 resistance mechanisms for therapeutic targeting in NSCLC

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Abstract

Immune checkpoint blockade is a promising treatment option for patients with non-small cell lung cancer (NSCLC), the most common type of lung cancer. Lung tumors harboring Kras/p53 (KP) mutations express higher levels of PD-L1 and respond better to anti-PD-1/-PD-L1 therapy than other Kras subsets[1]. However, despite the success of immunotherapy in treating late-stage lung cancer, tumors can gain acquired resistance through mechanisms that are not well understood.

Therefore, our goal was to elucidate the mechanisms of intrinsic and acquired anti-PD-(L)1 resistance in NSCLC. For this purpose, we developed several anti-PD-(L)1 sensitive and resistant mouse cell lines as working models. Preliminary studies suggest that our resistant cell lines do not exhibit known mechanisms of tumor cell-intrinsic immunotherapy resistance. To further identify genes that drive novel resistance mechanisms on a single cell resolution, we studied the differentially expressed genes (DEGs) between 344SQ (sensitive) and 344SQ^{PD1R1} (resistant) tumors treated *in vivo* with either IgG or anti-PD-1. After validation of numerous DEGs at both the mRNA and protein level, we obtained a list of candidate genes. Interestingly, we found methylthioadenosine phosphorylase (MTAP), a housekeeping gene known to play tumor-suppressor roles, to be consistently and significantly upregulated in anti-PD-1 resistant cell lines and tumors. *We hypothesized that gene expression changes in immunotherapy resistant tumor cells reprogram the tumor microenvironment to create an immunosuppressive milieu*. Our pilot *in vivo* study suggests that MTAP knockdown partially re-sensitizes 344SQ^{PD1R1} tumors to anti-PD-1 treatment and modulates intratumoral T cell activation status. The outcome of this project aims to provide novel therapeutic targets in combination with immunotherapy to overcome anti-PD-(L)1 resistance in NSCLC patients.

Background

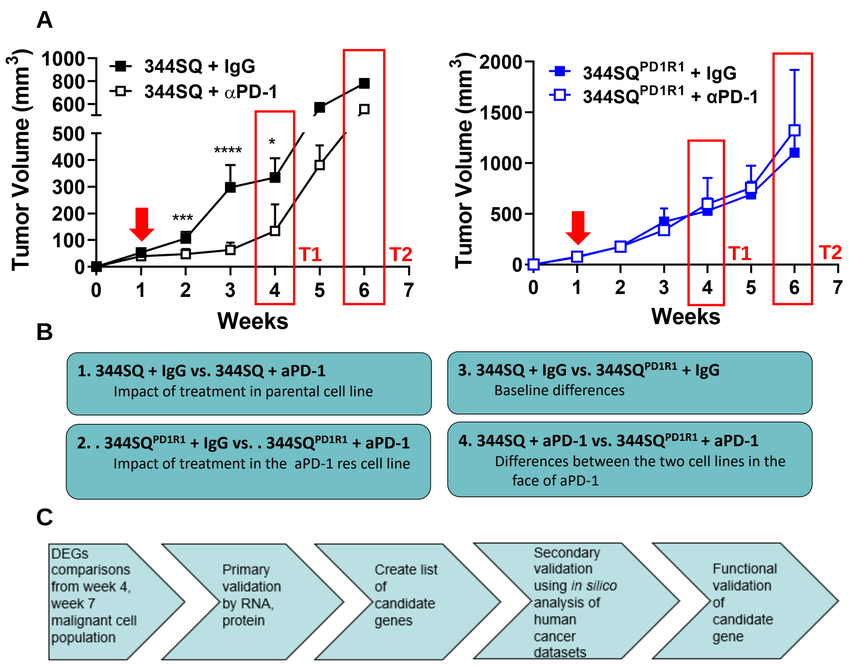

Figure 1. Single cell RNA-sequencing experimental design and validation. A. 344SQ (sensitive) or 344SQ^{PD1R1} (resistant) tumors were subcutaneously implanted into wildtype mice. One week post implantation, IgG or anti-PD-1 were i.p. injected weekly until end point (week 6). Tumors were collected at Time 1 (T1) (344SQ remained sensitive) and Time 2 (T2) (344SQ gained resistance) to be processed for single cell RNA-sequencing. **B.** Four comparisons between tumor models and treatments were performed on both T1 and T2 samples to obtain 8 sets of differentially expressed genes (DEGs) comparisons. **C.** Workflow of DEGs validation, identified from comparisons in Figure 1B.

Funding

CPRIT RP200235
CPRIT-MIRA RP160562-P3
NIH R37 CA214609
NIH F32 CA239292
2P50CA070907-21A

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Results

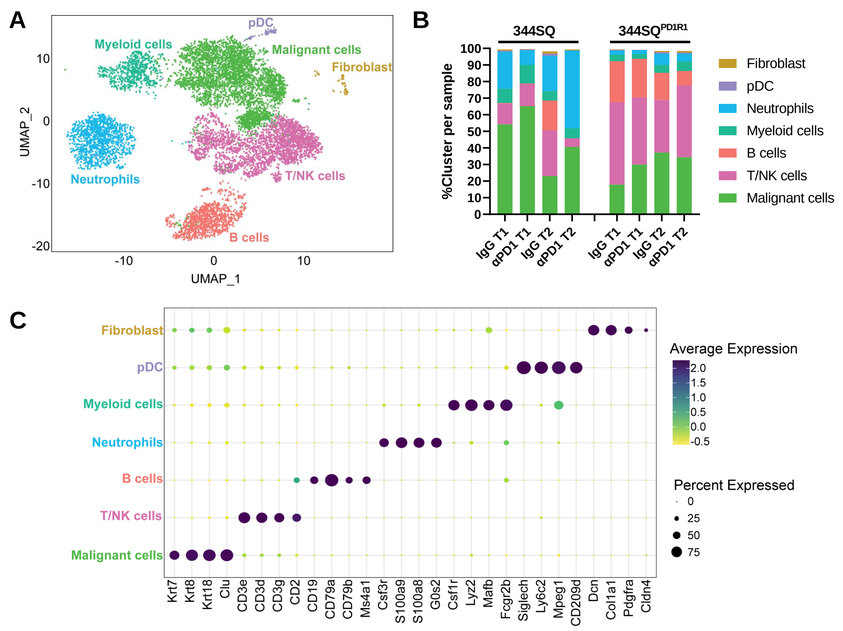

Figure 2. Clustering of single cells using markers from literature identified seven distinct populations. A. Unsupervised clustering of pooled samples identified 7 main cell types. **B.** Percentage of the identified cell clusters was graphed within each sample. **C.** Markers from literature used to identify each cluster.

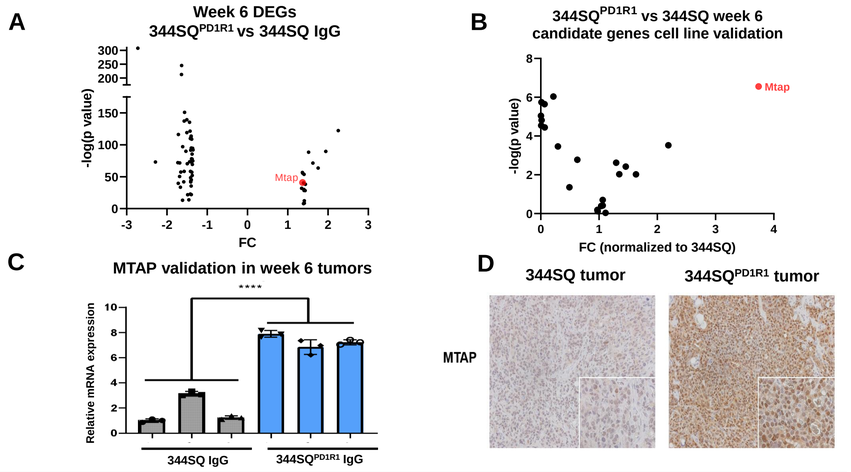

Figure 3. MTAP is significantly upregulated in resistant tumor in multiple scRNA-seq DEGs comparisons and its expression is validated. A. Volcano plot of week 6 344SQ^{PD1R1} vs 344SQ IgG DEGs comparison from which MTAP (red) was identified. MTAP was similarly upregulated in week 4 344SQ^{PD1R1} vs 344SQ IgG and aPD-1 treated DEGs comparisons (not shown). **B.** A list of candidate genes from T2 comparisons were validated by qPCR in cell lines, with MTAP being the highest upregulated in 344SQ^{PD1R1}. **C.** RNA validation of MTAP levels by qPCR in IgG treated week 6 344SQ^{PD1R1} and 344SQ tumors. **D**. Protein validation of MTAP levels by IHC in baseline SQ tumors.

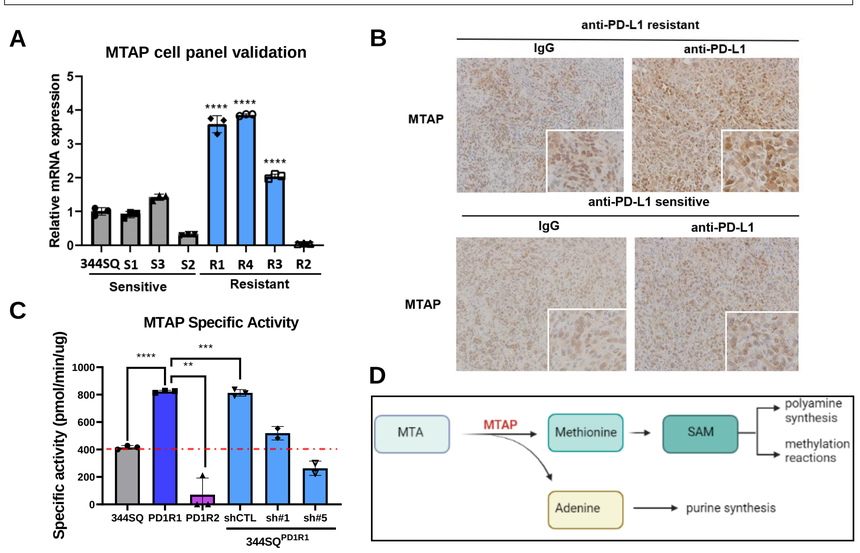

Figure 4. MTAP expression and enzymatic activity are further validated in different resistance models. A. Validation of MTAP RNA levels by qPCR from panel of 344SQ sensitive and resistant cell lines. **B**. IHC of MTAP in IgG/aPD-L1 treated tumors derived from sensitive/resistant KP GEMM cell lines. **C**. Assay based on MTAP (from cell lysate) conversion of MTA to adenine, which was converted to 8-dihydroxyadenine by xanthine oxidase. Absorbance read at 305nm for 30 min at kinetic mode[2]. **D**. Schematic depicting MTAP housekeeping function and downstream metabolites produced (made with BioRender).

Results

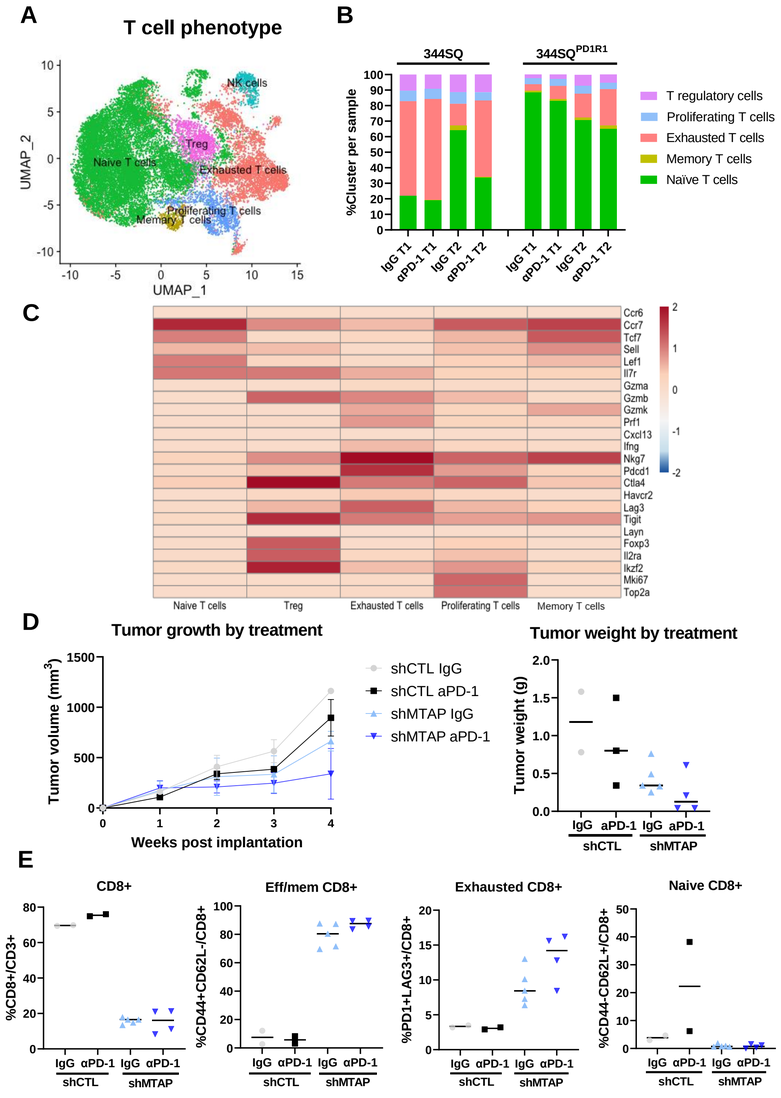

Figure 5. Pilot *in vivo* experiment shows MTAP KD in malignant cells partially re-sensitizes resistant tumors to anti-PD-1 treatment and alters T cell activation state. A. Clustering of T cell population from scRNA sequencing using markers from literature revealed 6 sub-populations with differential stages of T cell activation **B.** Percentage of each T cell sub-types out of total T cells for each sample. **C.** Markers used to identify each T cell subcluster phenotype. **D.** 344SQ^{PD1R1} shCTL, shMTAP tumor growth curves over 4 weeks (left). Tumor weights at week 4 end point (right). **E.** Flow cytometry of CD8+ T cells isolated from 344SQ^{PD1R1} CTL KD and MTAP KD tumors.

Conclusions

- We have developed and validated a working model to study anti-PD-(L)1 resistance in KP mutant lung cancer
- scRNA-sequencing analysis identifies high T/NK infiltration in the resistant tumor, but these T cells mostly remain at a naïve state
- Anti-PD-1 resistant model has significantly higher MTAP expression and enzymatic activity than the sensitive cell lines
- Pilot *in vivo* study suggests MTAP KD decreases tumor growth at baseline and partially re-sensitizes resistant tumors to anti-PD-1 treatment, potentially by pushing CD8+ T cell activation states closer toward an effector/memory phenotype

Future Directions

- Optimize the *in vivo* functional validation experiment with MTAP KD and MTAP OE cell lines to better characterize both the lymphoid and myeloid population in the tumor microenvironment
- Identify upstream regulators of MTAP expression in the context of anti-PD-1 resistance
- Further *in silico* analysis using human cancer datasets and *in vitro/in vivo* functional studies are needed to refine the list of candidate genes

References

1. Skoulidis et al. *Cancer Discovery*. 2015 August ; 5(8): 860–877.
2. Christopher et al. *Cancer res.* 2002**;** *62*(22), 6639–6644.