SMARCA4 inactivation drives aggressiveness in STK11/KEAP1 co-mutant lung adenocarcinomas through the induction of TGFβ signaling

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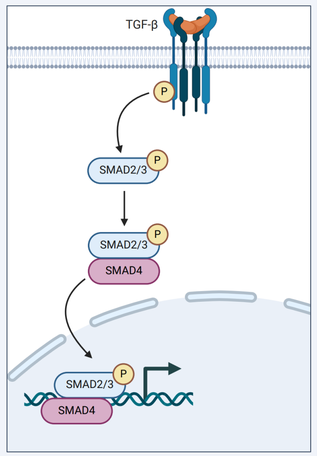
Introduction

Lung adenocarcinomas (LUADs), constituting 50% of lung cancers, are characterized by mutations in oncogenes such as KRAS or EGFR, known drivers of the disease. Though the development of driver-targeted therapies has significantly improved outcomes in subsets of LUAD patients, genomic analyses have shown that intra-driver genetic heterogeneity underlies the diversity of patient responses to these therapies and dramatically impacts patient survival.

Co-occurring alterations in the genes SMARCA4, STK11, and KEAP1 predict exceptionally poor prognosis. Studies exploring the molecular features of SMARCA4-deficient and STK11/KEAP1 (SK) co-mutant LUAD have yielded translational insights specific to these subsets, establishing a rationale to characterize the unique biology of SMARCA4/STK11/KEAP1 (SSK) triple-mutant LUAD.

Conclusions

Our data suggest that SMARCA4 inactivation leads to upregulation of TGFβ signaling in LUAD tumors with STK11 and KEAP1 inactivating mutations, thereby inducing an aggressive phenotype characterized by induction of pro-metastatic and stemness features. These results provide mechanistic insight into the aggressiveness of SSK triple mutant LUADs and nominates TGFβ signaling inhibition as a therapeutic target for SSK triple mutant LUADs.



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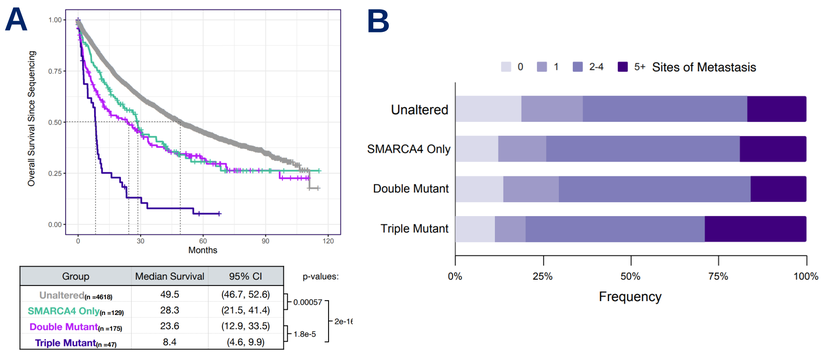
Results

STK11/KEAP1/SMARCA4-mutant LUADs are associated with poor survival and increased metastasis incidence



Group	Median Survival	95% CI
Unaltered(n =4618)	49.5	(46.7, 52.6)
SMARCA4 Only(n =129)	28.3	(21.5, 41.4)
Double Mutant(n =175)	23.6	(12.9, 33.5)
Triple Mutant(n =47)	8.4	(4.6, 9.9)

p-values: 0.00057, 1.8e-5, 2e-16

Figure 1. (A) Kaplan-Meier curves of overall survival for each cohort group, with median overall survival shown. P-values were calculated by the Mantel–Cox log rank test and match the pairwise comparisons indicated by brackets. CI = Confidence Interval. (B) Stacked bar plots depicting how frequently patients in each cohort group have 0, 1, 2-4, or 5+ reported sites of metastasis.

Transcriptomic analysis of triple-mutant versus STK11/KEAP1-mutant LUAD

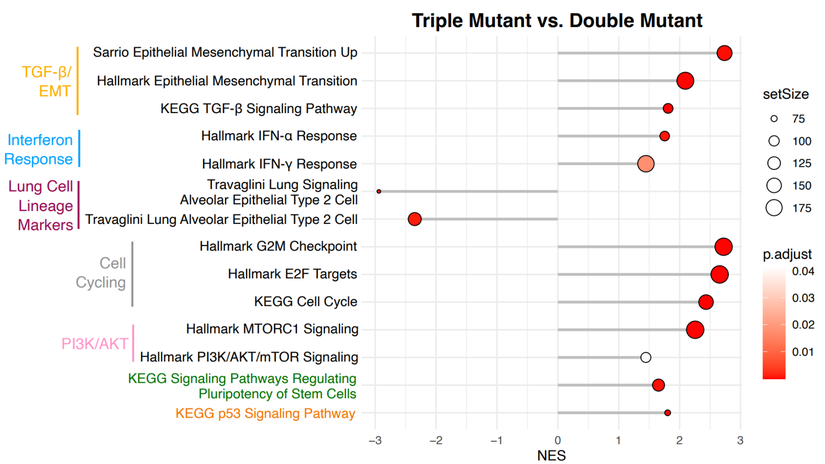


Figure 2. Gene Set Enrichment Analysis (GSEA) of differentially expressed genes (DEGs) between the Triple Mutant vs. Double Mutant LUAD clinical sample groups. All pathways shown are significantly enriched (adjusted p<0.05) and from the KEGG, GO, and mSigDB gene set databases. NES = normalized enrichment score

Triple STK11/KEAP1/SMARCA4 inactivation induces differential pro-oncogenic effects

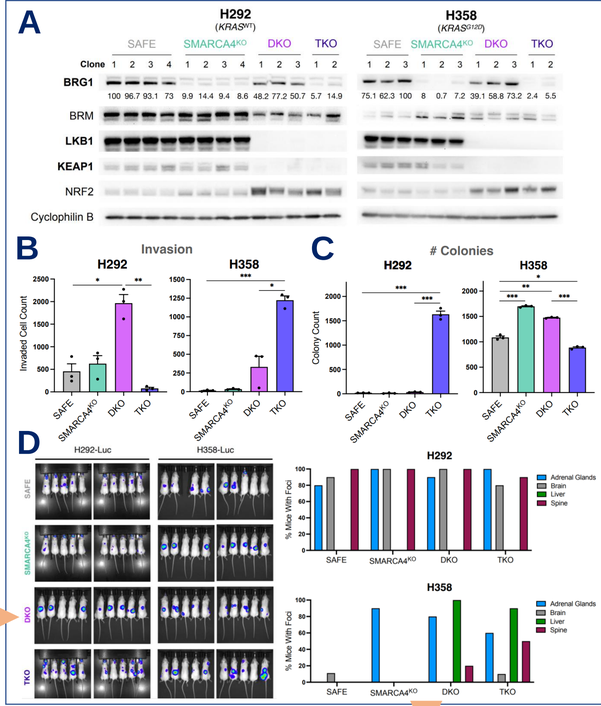


Figure 3. (A) Western blots of expression of CRISPR/Cas9 knockout (KO) proteins (bolded) and related proteins in the H292 and H358 single cell clone-derived cell line models. SAFE = sgSAFE.. Cyclophilin B was probed as a loading control. (B) *In vitro* invasion assays. Cell numbers shown are the sum of migrated or invaded cells counted in 5 image fields per replicate, with 3 technical replicates per genotype. Data are represented as mean ±SEM. Significance was calculated by a two-sample unpaired T-test with Bonferroni correction for 6 pairwise comparisons; (C) *In vivo* intracardiac injections. Metastasis imaging (day 21, left) and quantification per organ (right)

Oncogenicity in triple mutant LUAD is driven by TGFβ signaling

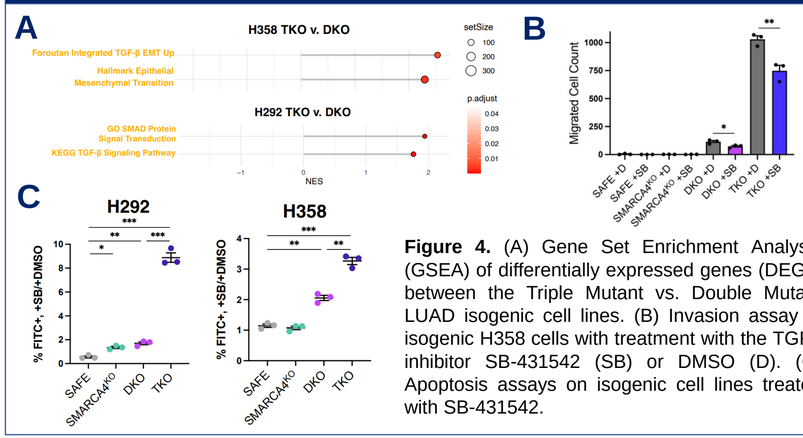


Figure 4. (A) Gene Set Enrichment Analysis (GSEA) of differentially expressed genes (DEGs) between the Triple Mutant vs. Double Mutant LUAD isogenic cell lines. (B) Invasion assay in isogenic H358 cells with treatment with the TGFβ inhibitor SB-431542 (SB) or DMSO (D). (C) Apoptosis assays on isogenic cell lines treated with SB-431542.