Smarca4-deficient lung cancers display a metastatic-like cell state and a distinct cell-of-origin

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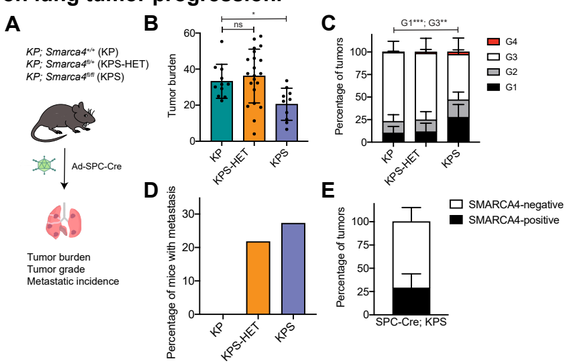
Abstract

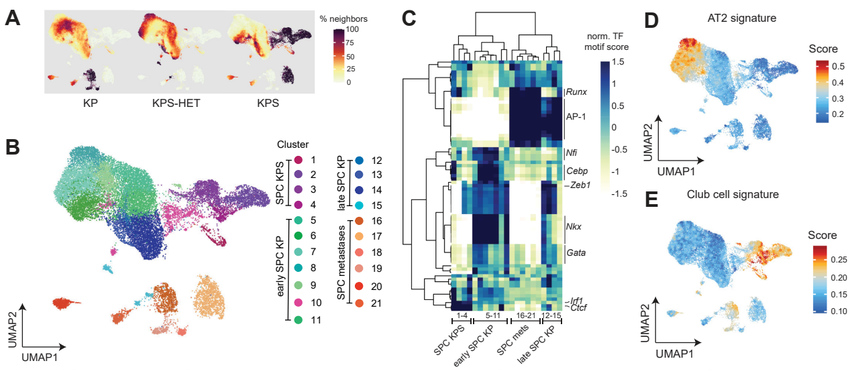
SMARCA4 (BRG1) is one of two mutually exclusive ATPases of SWI/SNF chromatin remodeling complexes, and is among the most frequently mutated genes in lung adenocarcinoma. Yet, the functional consequences of its alterations on tumor initiation and progression, the chromatin landscape, and gene expression in lung cancer are unknown. Here, we address these using a combination of genetically engineered mouse models, and epigenomic and transcriptomic profiling. By inactivating Smarca4 in an autochthonous mouse model of lung adenocarcinoma, we show that loss of Smarca4 sensitizes a distinct population of cells within the lung to malignant transformation and results in highly advanced tumors and increased metastatic incidence. Consistent with this phenotype, Smarca4-deficient tumors are absent of lung lineage transcription factor activities, similar to a metastatic cell state. Finally, by performing GeoMx digital spatial profiling using the mouse whole transcriptome atlas (Mu-WTA), we identify gene expression programs that characterize highly advanced and metastatic Smarca4-deficient lung cancers. Collectively, this work provides key insights into Smarca4-mediated tumor suppression and SWI/SNF function in the lung. GeoMx Assays are for RESEARCH USE ONLY. Not for use in diagnostic procedures.

Figure 1. *Smarca4* inactivation has divergent effects on lung tumor progression.



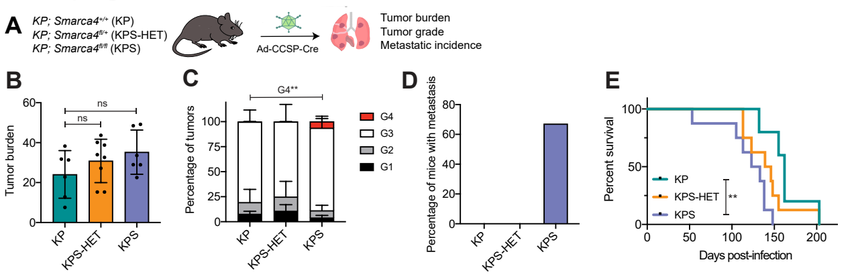
(A) Schematic diagram of the experimental strategy of tumor initiation in SPC-expressing cells in the lungs of KP, KPS-HET and KPS animals using adenoviral SPC-Cre (Ad-SPC-Cre), and subsequent analysis 17 weeks post-infection. **(B)** Quantification of tumor burden (% tumor area/lung area) in KP, KPS-HET and KPS animals 17 weeks post-infection. Data are mean ± s.d. **adjusted p-value* = 0.0443. **(C)** Distribution of histological tumor grades (% tumor grade area/total tumor area) in KP, KPS-HET and KPS animals 17 weeks post-infection. Data are mean ± s.d. ****adjusted p-value* = 0.0009 ***adjusted p-value* = 0.0013. **(D)** Percentage of KP, KPS-HET and KPS mice with metastasis 17 weeks post-infection. Chi-square: ns, $p = 0.1672$. **(E)** Quantification of the percentage of SMARCA4-postive and –negative tumors in the lungs of KPS animals infected with Ad-SPC-Cre. Data are mean ± s.d.

Figure 2. Epigenetic states in *Smarca4*-deficient primary tumors resemble metastases.



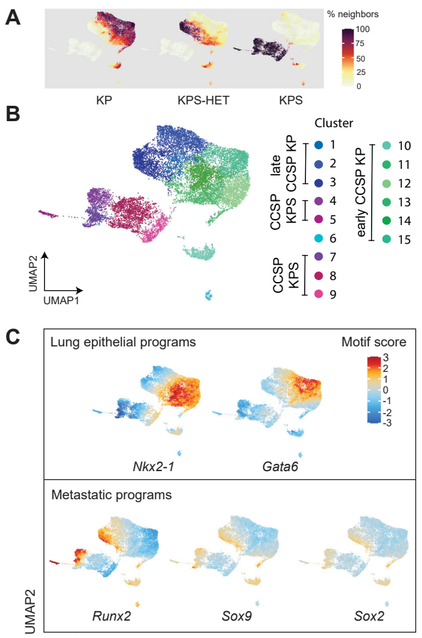
(A) UMAP visualization of single-cell chromatin accessibility profiles of 25,229 cancer cells isolated from primary tumors and metastases of KP, KPS-HET and KPS animals colored by % of cell neighbors per genotype. **(B)** UMAP visualization of single-cell chromatin accessibility profiles colored by cluster. **(C)** Marker motif scores for each cluster. UMAP visualization of dataset colored by **(D)** AT2 and **(E)** club cell gene signature scores.

Figure 3. *Smarca4* inactivation in tumor-initiating CCSP$^+$ cells accelerates tumor progression.



(A) Schematic diagram of the experimental strategy of tumor initiation in CCSP-expressing cells in the lungs of KP, KPS-HET and KPS animals using adenoviral CCSP-Cre (Ad-CCSP-Cre), and subsequent analysis 16 weeks post-infection. **(B)** Quantification of tumor burden (% tumor area/lung area) in KP, KPS-HET and KPS animals 16 weeks post-infection. Data are mean ± s.d. **(C)** Distribution of histological tumor grades (% tumor grade area/total tumor area) in KP, KPS-HET and KPS animals 16 weeks post-infection. Data are mean ± s.d. ***adjusted p-value* = 0.0044. **(D)** Percentage of KP, KPS-HET and KPS mice with metastasis 16 weeks post-infection. Chi-square: $p = 0.0185$. **(E)** Survival curves of KP, KPS-HET and KPS animals infected with Ad-CCSP-Cre through intratracheal instillation. Log-rank test (KP vs KPS): **$p = 0.0086$.

Figure 4. The epigenetic states of *Smarca4*-deficient primary tumors are driven by *Smarca4* loss.



(A) UMAP visualization of single-cell chromatin accessibility profiles of 16,321 cancer cells isolated from CCSP-Cre-initiated primary tumors of KP, KPS-HET and KPS animals colored by % of cell neighbors per genotype. **(B)** UMAP visualization of scATAC-seq profiles colored by cluster. **(C)** UMAP visualization of the scATAC-seq dataset colored by lung lineage and metastatic program motif scores.

Acknowledgements

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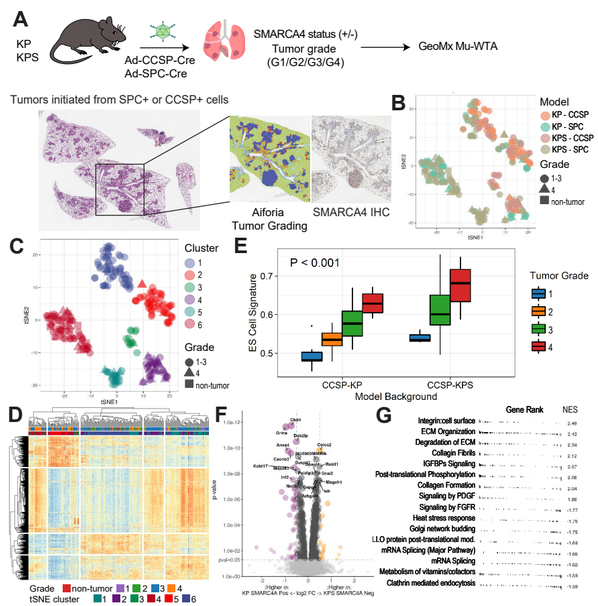
Figure 5. Distinct transcriptional profiles are enriched in advanced *Smarca4*-deficient tumors.



(A) Experimental strategy to perform digital spatial profiling on murine models of *Smarca4*-deficient LUAD to generate gene expression profiles characterizing cell-of-origin, tumor grade and SMARCA4 status (top panel). Representative tumor-bearing lung showing H&E staining, Aiforia tumor grading and SMARCA4 IHC (bottom panel). **(B)** tSNE visualization of gene expression profiles colored by genotype and cell-of-origin. **(C)** tSNE visualization of gene expression profiles colored by cluster. **(D)** Heatmap of marker genes of each cluster. **(E)** Embryonic stem cell-like signature (Wong et al., 2008) scores among the different samples. **(F)** Differential gene expression between CCSP-Cre-initiated KP Grade 3 tumors compared to CCSP-Cre-initiated KPS Grade 3 tumors. **(G)** Gene set enrichment analysis of differential genes between CCSP-Cre-initiated KP Grade 3 tumors compared to CCSP-Cre-initiated KPS Grade 3 tumors.

Future Directions

- Determination of the mechanism behind the differential sensitivities of SPC$^+$ and CCSP$^+$ to malignant transformation upon *Smarca4* loss
- Functional validation of downstream modulators of *Smarca4*-mediated tumor suppression in the lung
- Identification of potential therapeutic strategies based on expression profiles

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