#1138 A single-cell spatially resolved atlas of immune-mediated control of lung adenocarcinoma

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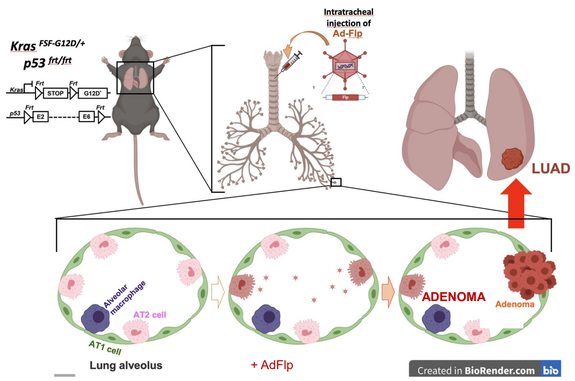
Background

Lung adenocarcinoma is one of the leading causes of cancer-related mortality. One of the most frequently occurring mutations in lung cancer is KRAS mutations. Recent development of chemical inhibitors that specifically target oncogenic variants of Ras, particularly the commonly mutated KRAS-G12D isoform, represents a significant breakthrough in targeted therapeutics. The tissue-level mechanisms underlying the cell-autonomous and non-cell-autonomous effects of KRas-G12D inhibitors are poorly understood. Additionally, the effectiveness of KRas-G12D inhibitors in lung cancer models remains unknown. To address these gaps in knowledge, we analyzed the spatial interactions between cancer cells and the surrounding tissue microenvironment during the process of tumor eradication mediated through KRas-G12D inhibitors.

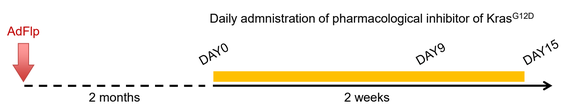
Methods

Genetic mouse model of non-small cell lung cancer (NSCLC)



12-week-old Kras$^{FSF-G12D/+}$, p53$^{frt/frt}$ mice received one intratracheal instillation of adenoviral particles serotype 5, an established tool to target epithelial cells of the lung encoding Flp recombinase[1,2,3]. Activation of mutant Kras^{G12D} expression and KO of p53 trigger the formation of multifocal adenomas from AT2 cells in 2-3 weeks and of LuADs in 2 months.

Pharmacological treatment and tissue processing



Starting two months after AdFlp instillation, mice received daily injections of a pharmacological inhibitor of mutant Kras. Control mice were euthanized at Day 0 (n=6), and treated mice were sacrificed after 9 or 15 days of treatment (n=5). Lungs were perfused with PBS, fixed in 4% PFA for 24 hours and room temperature and paraffin embedded. 2 consecutive 4µm-thick sections were used for H&E staining and CosMx SMI 1,000-plex RNA assay.

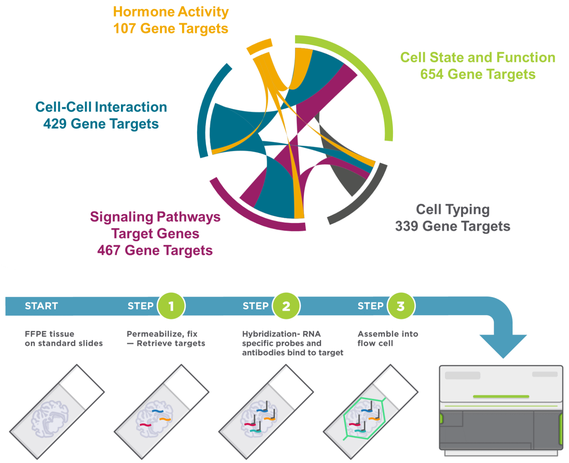
References:

1. DuPage, M., Dooley, A. L. & Jacks, T. Nat Protoc 4, 1064-1072 (2009)
2. Lee CL, Moding EJ, Huang X, Li Y, Woodlief LZ, Rodrigues RC, Ma Y, Kirsch DG. Dis Model Mech. (2012)
3. Meuwissen, R., Linn, S. C., van der Valk, M., Mooi, W. J. & Berns, A. Oncogene 20, 6551-6558 (2001)

Methods

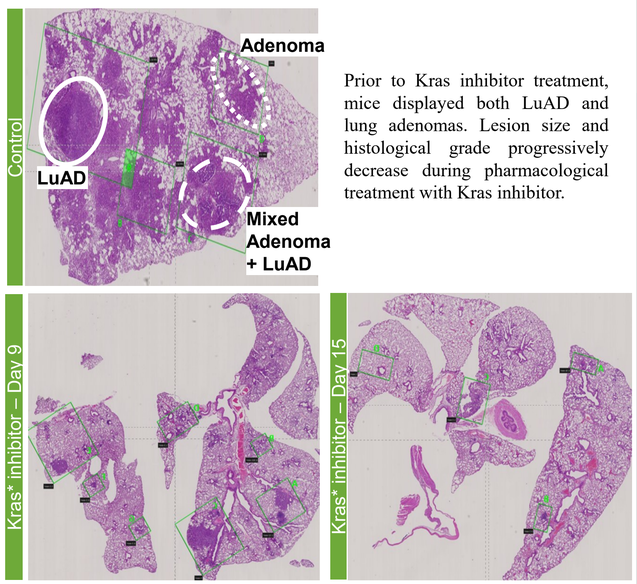
CosMx SMI assay with 1,000-plex Mouse Universal Cell Characterization panel

CosMx SMI 1,000-plex Mouse Universal Cell Characterization panel is the highest-plex RNA panel with wide coverage of key marker genes in immunology, signaling transduction and cell response to stimulation during oncology process



Results

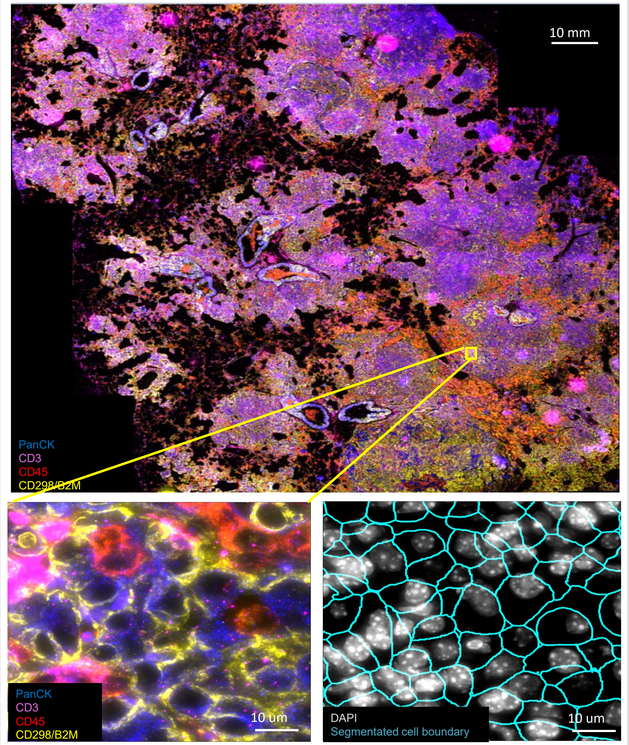
H&E reveals effective pharmacological treatment



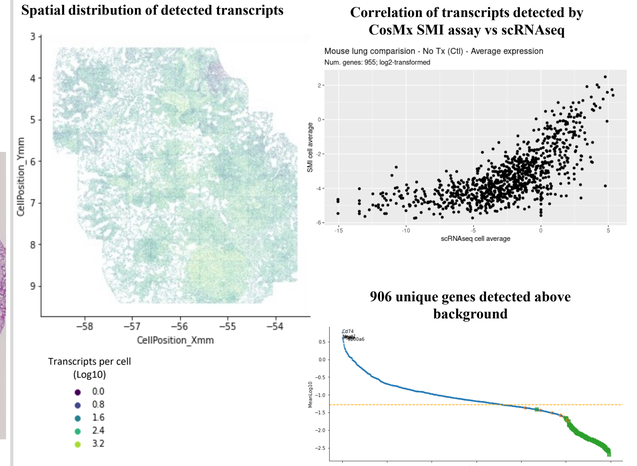
Prior to Kras inhibitor treatment, mice displayed both LuAD and lung adenomas. Lesion size and histological grade progressively decrease during pharmacological treatment with Kras inhibitor.

Results

Protein markers stain on the same slides as RNA assay ensures state-of-art segmentation quality



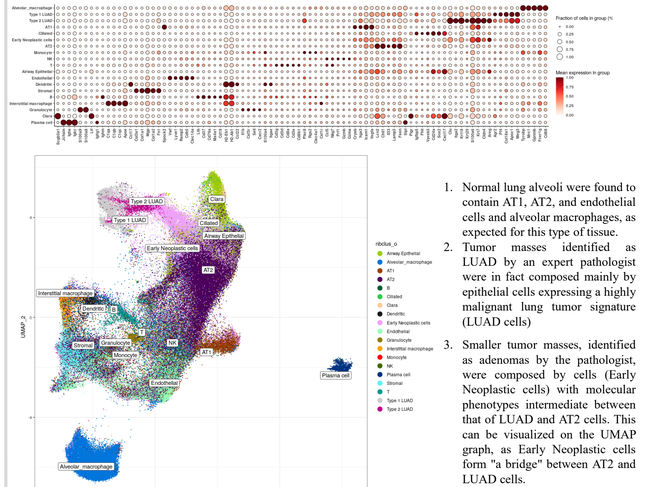
High sensitivity and specificity of spatial transcripts detection



Results

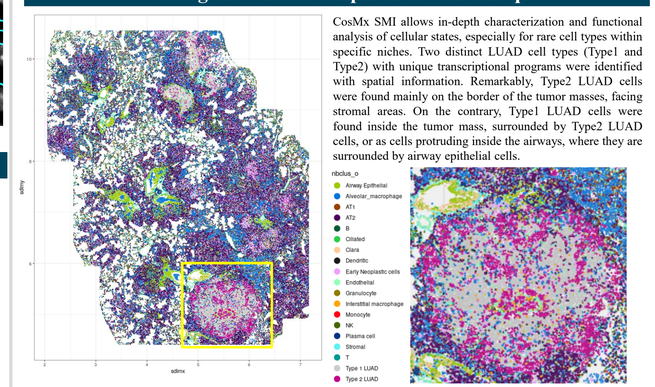
CosMx SMI results match "gold standard"

Cell-typing using CosMx technology revealed all major cell types identified by single-cell RNA-seq performed on the same samples using droplet-based technology (10X), with accurate spatial location.



1. Normal lung alveoli were found to contain AT1, AT2, and endothelial cells and alveolar macrophages, as expected for this type of tissue.
2. Tumor masses identified as LUAD by an expert pathologist were in fact composed mainly by epithelial cells expressing a highly malignant lung tumor signature (LUAD cells)
3. Smaller tumor masses, identified as adenomas by the pathologist, were composed by cells (Early Neoplastic cells) with molecular phenotypes intermediate between that of LUAD and AT2 cells. This can be visualized on the UMAP graph, as Early Neoplastic cells form "a bridge" between AT2 and LUAD cells.

High-plex single-cell spatial assay allows deeper understanding of biological context compared to scRNAseq



CosMx SMI allows in-depth characterization and functional analysis of cellular states, especially for rare cell types within specific niches. Two distinct LUAD cell types (Type1 and Type2) with unique transcriptional programs were identified with spatial information. Remarkably, Type2 LUAD cells were found mainly on the border of the tumor masses, facing stromal areas. On the contrary, Type1 LUAD cells were found inside the tumor mass, surrounded by Type2 LUAD cells, or as cells protruding inside the airways, where they are surrounded by airway epithelial cells.

Conclusion

This study provides novel insights into the temporal and spatial dynamics of KRas-G12D inhibitor-mediated tumor regression in lung cancer, shedding light on the previously unknown cell-cell interactions occurring during this process. By investigating these spatiotemporal aspects, we aim to enhance our understanding of lung cancer biology and potentially identify new immunotherapeutic biomarkers. Moreover, CosMx SMI assay is a powerful research tool and have great implications for the design of future preclinical studies exploring the potential of immuno-oncology combination therapies.