Spatial Analysis of Single Cell Ligand-Receptor Expression Elucidates Tumor-Immune interactions in NSCLC

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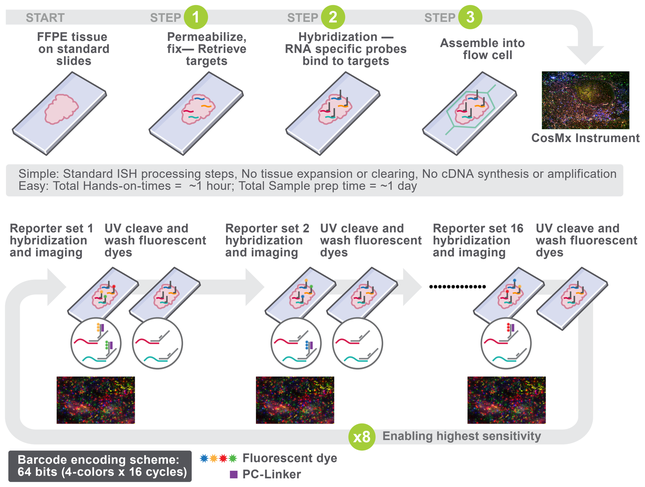
Abstract

Tumors may utilize immunomodulatory ligand-receptor (LR) signaling to evade immune surveillance and destruction. **CosMx™, a single cell spatial transcriptomics technology, has enabled us to infer ligand-receptor signaling across millions of physically interacting cells**. Using CosMx data from non-small cell lung cancer (NSCLC), we search for evidence of coordinated ligand-receptor expression in neighboring tumor and immune cells.

CosMx Overview

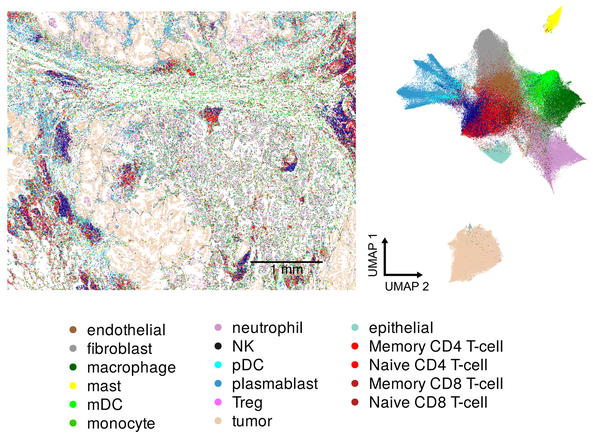
a



a Description of sample prep for CosMx followed by reporter probe readout methods for Spatial Molecular Imaging

1. Cell Typing in FFPE NSCLC

b



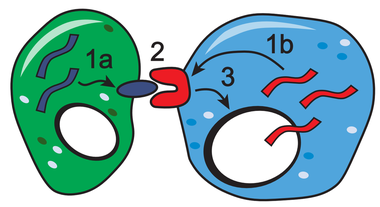
b Cell typing in NSCLC from one of 5 tumors (Lung 5) displayed in tissue space (left) and UMAP space (right)

The CosMx 980 plex Universal Cell Characterization (UCC) panel enables robust identification of cell types in within the tumor microenvironment. **The spatial locations of each of these cell types is preserved, allowing us to observe interactions between cell types.** In the CosMx NSCLC dataset:

- 18 cell types can be identified (**fig b**)
  - o 3 stromal cell types
  - o 14 immune cell types including 5 T cell subtypes
- Cell types are spatially segregated into tumor, immune, and stromal compartments
- Regions of **tumor cells shows low levels of T cell and macrophage infiltration**
- See **Poster #106** for more information about this dataset

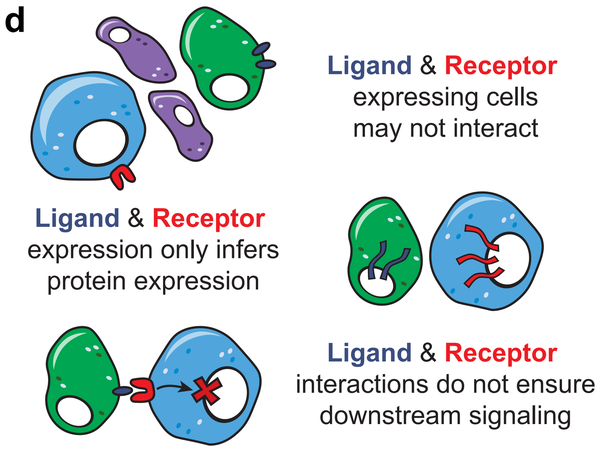
2. Measuring LR Signaling

c



1. **Ligand** & **Receptor** RNA is Translated into protein
2. **Ligand** & **Receptor** form a complex
3. Signaling impacts **Receptor** expressing cell

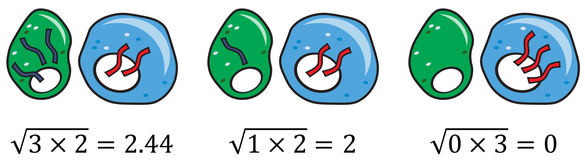
d



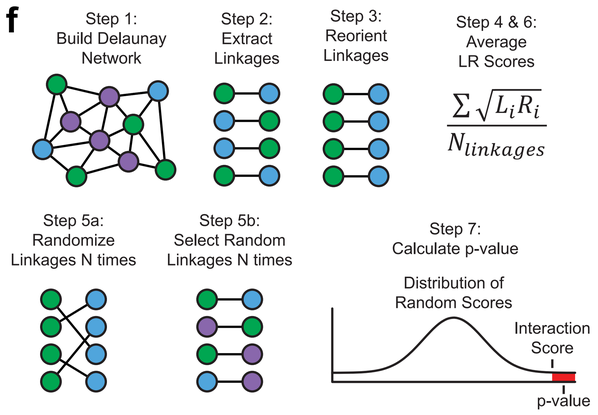
c Ligand-Receptor signaling starts with RNA expression, continues with translation, protein binding, and finally downstream signaling
d Ligand-Receptor signaling can be difficult to measure when using existing gene expression technologies

e

$$[LR_{eq}] = K_D{}^{-1}[L_{eq}][R_{eq}] \qquad \sqrt{L \times R} = Score$$



f



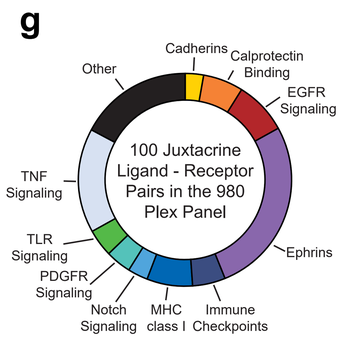
e Ligand-Receptor binding kinetics and signaling can be represented by scoring ligand and receptor co-expression between interacting cells pairs
f Ligand-Receptor interactions are scored across the desired cell to cell linkages extracted from a spatial adjacency network. The average linkage score is subsequently tested against a null distribution of N sets of randomly simulated and/or sampled linkages

The single cell spatial information provided by CosMx allows us to score LR co-expression between neighboring cells. As with scRNAseq, such scores may only indicate the potential for LR signaling. However, **the added spatial element eliminates the possibility of falsely identifying LR mediated communication between cell types that do not physically interact *in situ***. Using spatial information, we may also infer LR interactions between cell types that are enriched:

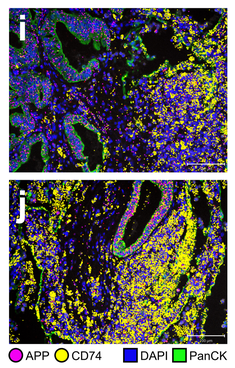
- By the spatial arrangement of those cells
- Relative to all other cell-to-cell interactions in the sample

3. LR Signaling in NSCLC

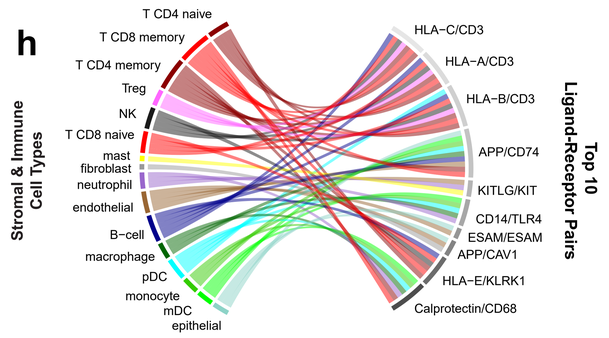
g



i, **j**

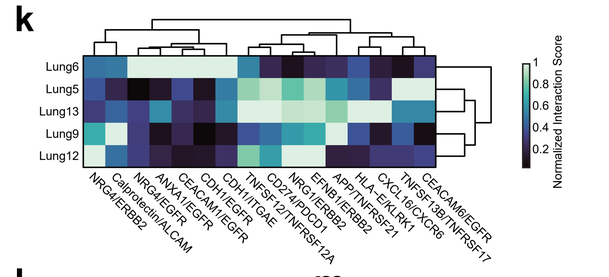


h



g 100 juxtacrine ligand-receptor pairs are included in the UCC panel
h Chord diagram between all non-tumor cell types and the top 10 enriched LR pairs. Each chord indicates an LR pair that was enriched in linkages where the connected cell type was the receptor expressing cell.
i, j Immunofluorescence (IF) images for two field of views in Lung 5 where detected RNAs for the LR pair APP/CD74 are plotted atop the image

k



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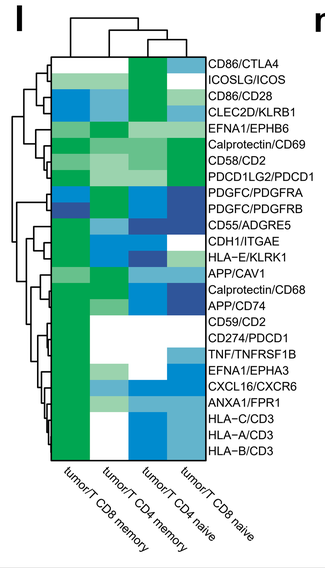
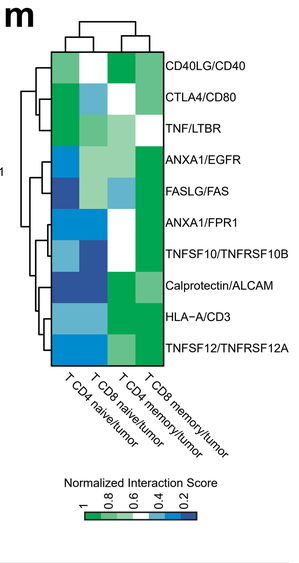


m



k Heatmap of average LR scores for all spatially enriched tumor to T cell interactions across all 5 tumors. **l, m** Heatmap of LR scores enriched in at least one type of tumor to T cell or T cell to tumor interaction respectively. All heatmaps are scaled with a maximum of 1 for each LR pair.

The CosMx UCC panel contains many LR pairs, including 100 for juxtacrine signaling. Of these, many are enriched in sets of interactions grouped by the cell type of the receptor expressing cell. (**fig h**) In the top 10 enriched of these pairs, **MHC-I to CD3 interactions are enriched in T cells**. Additionally, CD14/TLR4 is enriched in myeloid cell types while ESAM and CAV1 interactions are enriched in endothelial cells.

3. LR Signaling in NSCLC (cont.)

Using the methods described in (**fig f**) we find 16 LR pairs are significantly enriched between interacting tumor cells and T cells. **PD-L1/PD-1 (CD274/PDCD1), a known contributor to T cell exhaustion, is enriched** here and shows variation across the tumors in the NSCLC dataset. (**fig k**)

Furthermore, PD-L1/PD-1 and CD86/CTLA4 are respectively enriched in tumor to CD8 memory and CD4 naïve T cell interactions. (**fig l**) Lastly, apoptosis-inducing interactions such as FASLG/FAS and TNFSF10/TNFRSF10B are enriched in CD8 memory (effector) T cell to tumor interactions. (**fig m**)

Methods

Delaunay triangulation was used to build a spatial adjacency network of all cells. For each interacting cell pair, we calculated an LR score using the geometric mean of their ligand and receptor expression. (**fig e**) This LR score was recalculated for all 100 juxtacrine LR pairs. Next, these scores were grouped and averaged either by the receptor expressing cell type or by each cell type pair that included T cells. Finally, **each average score was tested to determine if it was enriched by its unique interaction type or by the spatial arrangement of all cells**. This was done by producing a null distribution of simulated average scores calculated using randomized adjacency networks or by randomly sampling sets of cell pairs. (**fig f**)

Conclusions

CosMx fuses high plex single cell expression data with detailed spatial information about each cell. This enables us to measure potential LR mediated interactions with unprecedented depth. Using the CosMx UCC panel to probe 5 NSCLC tumors in conjunction with our LR scoring system we observed:

- How LR signaling varies between tissues
- MHC-I to CD3 interactions are enriched in T cells
- Immune-suppressive signaling between tumors and T cells, including PD-L1/PD-1 and CD86/CTLA4
- Apoptotic signaling between T cells and tumor cells

Overall, our methods and LR scoring system recapitulate known biology while providing a unique avenue for decoding novel cellular communication networks.

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