#156 Spatial Transcriptomic signatures of the fundamentals of immune oncology

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Abstract

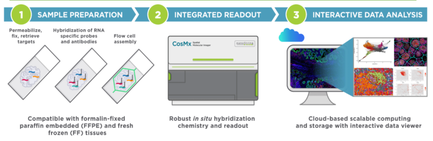
Spatial transcriptomics platforms produce immense datasets, measuring hundreds to thousands of genes across up to 1 million single cells in a single tissue. For investigators interested in clinical outcomes, for example of responders vs. non-responders to an immunotherapy, this data richness presents as not a windfall but a quagmire. To facilitate immune-oncology research, we have devised algorithms for automatically measuring the fundamental units of the tumor-immune interaction in spatial transcriptomics data. Given data from a single tumor, our algorithms output dozens of relevant, human-intelligible variables, which we propose as ideal outputs for multi-tumor comparisons.

Our first set of algorithms is ***knowledge-driven***, measuring outputs that the field already knows to be important. These variables cover anti-tumor immune activities like cytotoxicity and antigen presentation, tumor-intrinsic processes like cell proliferation and hypoxia response, and immunosuppressive tumor activities like immune checkpoint expression.

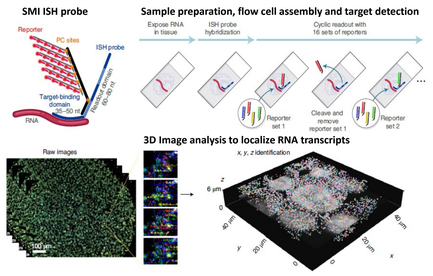
A second set of algorithms is ***data-driven***. We identify modules of immune-signaling genes with tendencies to be expressed in the same locations, and we quantify these modules across the space of a tumor. An example output quantifies hotspots of a module, COL1A2-LUM, consisting of CD276, CDH11, COL12A1/2, COL12A1, COL3A1, COL5A1, COL5A2, IGF2, LUM, MEG2. A module, C10A-C10B, includes genes of CD74, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DRB1 which are marker genes of MHC2 signature. A module of IGHD-CD37 includes CCL19, CD19, CD37, CD40, CD79A, IGHD and IL16: part of these genes is marker genes of Tertiary Lymphoid Structure.

In summary, our spatial signatures – currently 13, with more under development – measure tumor attributes fundamental to anti-tumor immunity and immune evasion. We propose them as a core set of variables for describing relationship between tumor and tumor microenvironment. For further application, these metrics can be used as a signal of patient's disease progress or treatment response in immunotherapy.

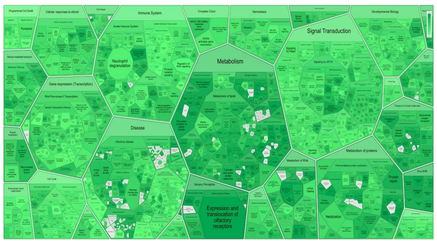
Overview of the CosMx™ SMI



RNA data collection



CosMx™ 6,000-plex panel coverage of biological pathways



Signatures of Immune Oncology

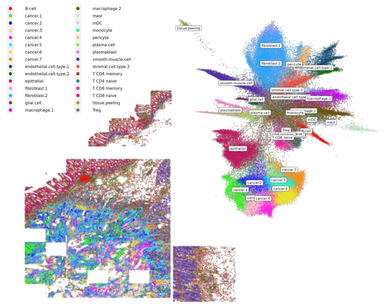
Signatures	Category	Description	Cell Types	Genes
Antigen Presenting Machinery	Tumor immunogenicity	measures the abundance of genes in the MHC class I antigen presentation pathway and some key genes involved in processing the antigens prior to presentation	Tumor, Dendritic Cells	B2M, HLA-A, TAP1, TAP2, CD81, LAG3
Apoptosis	Tumor regulation	captures genes associated with apoptotic(cell death) processes.	Tumor	BAX, BCL2L1
Cytotoxicity	Anti-tumor Immune activity	measure the molecules used by NK and CD8+ T cells	T-cells, NK cells	GNLY, GZMA, GZMB, GZMH, PRF1
Glycolytic Activity	Metabolism		Tumor	AKT1, ENO1, GLUD1, HIF1A, LDHA, SLC2A1, TPI1
Hypoxia	Inhibitory Metabolism	measures genes associated with reduced oxygenation in the tumor	All	SLC2A1
IFN Downstream Signaling	Anti-viral immune activity		Tumor, multiple	IFI27, IFI6, IFIH1, IFIT1, IFIT3, IFITM1, ISG15, MX1, OAS1, OAS2
IFN_Gamma_Signaling	Anti-tumor Immune activity	Tracks the canonical response to Type II interferon, including the most universal components of that response.	macrophage, NK cells, Tumor	CXCL10, CXCL9, STAT1
Inflammatory Chemokines	Inhibitory Immune Signaling	recuiting monocytes, neutrophils and other effector cells from the blood to sites of infction or tissue damage such as the tumor microenvironment	neutroils, monocytes, leukocytes	CCL2, CCL4, CCL8
Lymphoid	Anti-tumor Immune activity	immune aggregates with varying degrees of organization in response to chronic inflammation or infection.	T-cells, B-cells, dendritic cells	CD2, CD27, CD38, CD3D, CD3E, CD3G, CD40LG, CD48, CD79A, CD8A, CD8B, CTLA4, CX3CL1, CXCL10, CXCL13, CXCL9, CXCR3, EOMES, GNLY, GZMA, GZMB, GZMH, GZMK, ICOS, IDO1, IFITM1, IFNG, IGF2R, IL2RG, IRF4, JAK1, JAK2, KLRB1, KLRK1, LAG3, MS4A1, PDCD1, PRF1, STAT1, TBX21, TIGIT
MHC2	Anti-tumor Immune activity	measures the major human leukocyte antigens (HLA) involved in MHC Class II antigen presentation.	dendritic cells, macrophage, B-cells	CD74, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DRB1
Myeloid Inflammation			macrophage, myeloid cells	AREG, CCL20, CSF3, CXCL1, IER3, IL6, PTGS2
Proliferation	Tumor regulation		Tumor	CENPF, MKI67, UBE2C
Tumor Inflammation Signature	Anti-tumor Immune activity	measures the abundance of a peripherally suppressed adaptive immune response within the tumor	Tumor, T cells, NK cells, dentritic cells	CCL5, CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, IDO1, LAG3, NKG7, PDCD1LG2, STAT1, TIGIT
Tertiary Lymphoid Structure (TLS)	Anti-tumor Immune activity	immune aggregates with varying degrees of organization in response to chronic inflammation or infection.	T-cells, B-cells, dendritic cells	CD19, CD20, CETP, CCR7, SELL, LAMP3, CCL19, CXCL9, CXCL10, CXCL11, CXCL13, CD208, CD3

Methods

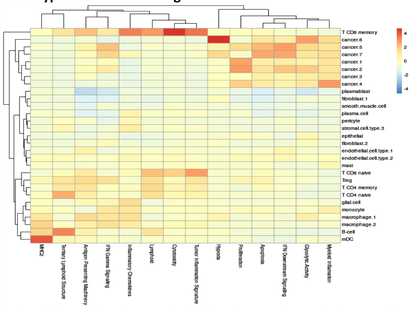
Our knowledge-driven approach scores single cells and cell neighborhoods for previously-derived metagenes. The data-driven method builds metagenes from spatially correlated sets of genes, identified using the InsituCor R package (Danaher *et al.*, "InSituCor: a toolkit for discovering non-trivial spatial correlations in spatial transcriptomics", manuscript under review).

Knowledge-Driven Method

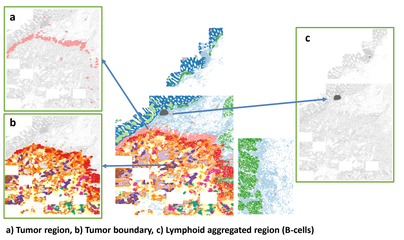
Spatial plot and UMAP colored by Cell types



Cell Type involvement in signatures

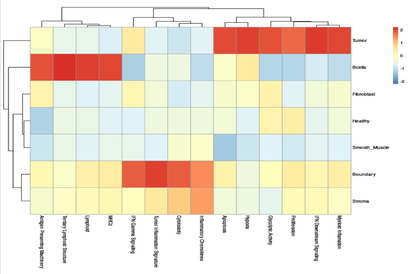


InsituType algorithm and additional manual annotation could define refined cell types including six cancerous sub cell types, immune cell types such as B cells, CD4/CD8 T cells, NK cells, Treg and smooth muscle cells. Hypoxia, Apoptosis, Glycolytic activity, IFN downstream signaling, and Myeloid inflammation signatures were strongly expressed in cancer cells. Immune cells shows high expression of Tertiary lymphoid structure, MHC2, Tumor inflammation signaling, Cytotocixity, and Lymphoid signatures.

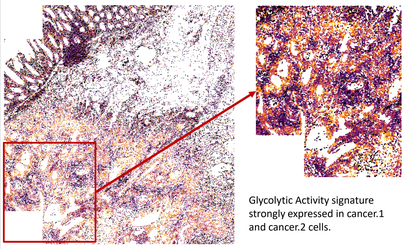


a) Tumor region, b) Tumor boundary, c) Lymphoid aggregated region (B-cells)

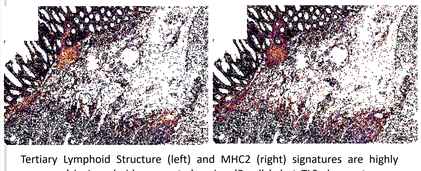
Niche involvement in signatures



Spatial clustering was conducted to define broad biological structures: tumor region, lymphoid aggregated region (B cells), tumor boundary region, stroma, smooth muscle, fibroblast. Myeloid inflammation, IFN downstream signaling, Proliferation, Hypoxia and Apoptosis are strongly expressed in the tumor regions. Cytotoxity and Tumor inflammation signature are expected to be expressed in T cells, NK cells or Dendritic cells which are cumulated in tumor boundary region. Tertiary lymphoid structure, Lymphoid and MHC2 are highly expressed in Lymphoid aggregated region which mainly consists of B cells.



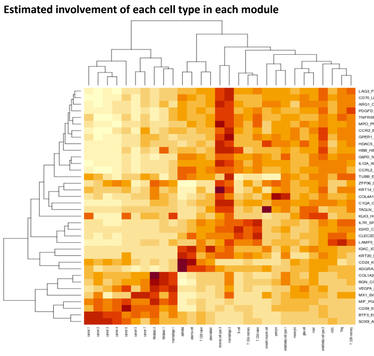
Glycolytic Activity signature strongly expressed in cancer.1 and cancer.2 cells.



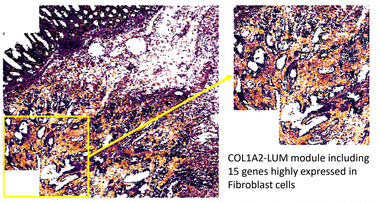
Tertiary Lymphoid Structure (left) and MHC2 (right) signatures are highly expressed in Lymphoid aggregated region (B-cells), but TLS shows stronger expression than MHC2 and MHC2 also shows its expression in Tumor boundary (T-cells).

Data-Driven Method

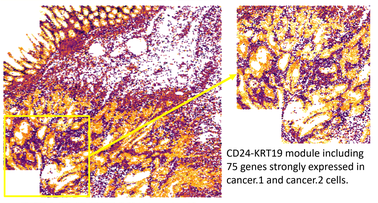
Estimated involvement of each cell type in each module



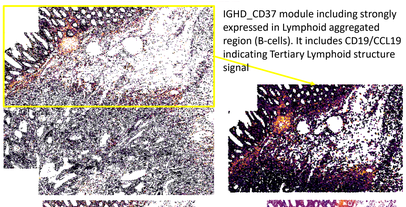
Above) The estimated score of each module including at least 6 genes is visualized by the cell types. Each module strongly expressed in different cell types. Following figures show three modules spatially differently distributed.



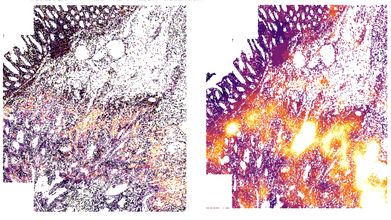
COL1A2-LUM module including 15 genes highly expressed in Fibroblast cells



CD24-KRT19 module including 75 genes strongly expressed in cancer.1 and cancer.2 cells.



IGHD_CD37 module including strongly expressed in Lymphoid aggregated region (B-cells). It includes CD19/CCL19 indicating Tertiary Lymphoid structure signal



(Above) MX1-ISG15 module including IFI6,IFIT3,MX1,OAS1/2,CXCL10,STAT1 is highly expressed in Tumor boundary and tumor region close to the boundary. This module's genes are marker genes of IFN downstream signaling and IFN Gamma signaling. (left)InsituCor cell-level score, (right) InsituCor environmental score

Conclusions

First, we defined 14 signatures with known its gene lists and its biological functions in oncology. The knowledge-driven method was applied to CosMx spatial RNA data to estimate each signature's score and its spatial distribution was evaluated. Most signatures were strongly expressed in its expected cell types, but a couple of signatures did not behaved as we expected. Tertiary lymphoid structure was highly expressed in lymphoid aggregated region (B-cells) and MHC2 was expressed in tumor boundaries and B-cells.

Second, we applied data-driven method using InsituCor R package developed by NanoString and captured about 28 gene-modules including at least 6 genes. These modules are automatically captured based on the conditional correlation structure. We observed that the identified modules could capture spatial structures better than the knowledge-driven method.

References

1. Danaher P, Zhao E, Yang Z, Ross D, Gregory M, Reitz Z, Kim TK, Baxter S, Jackson S, He S, Henderson DA. Insitutype: likelihood-based cell typing for single cell spatial transcriptomics. bioRxiv. 2022.10.19.512902; doi: https://doi.org/10.1101/2022.10.19.512902
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