Mapping the tumor landscape: Spatial Multi-omics analysis reveals immune and stromal heterogeneity across early and late-stage NSCLC

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Abstract

Using a combination of modalities such as spatial transcriptomics (STx) and sequential multiplex immunofluorescence (mIF) on different stages of non-small cell lung cancer samples we investigated spatial distribution, phenotype, function, and gene expression profile. In spite of immune cell presence stage IIIB tumor has evolved and adapted to thrive under pressure with upregulation of PD-L1 on epithelial cells leading to immune evasion. All three tumor types were found to have M2 macrophage population which is prognostically undesirable. The tertiary lymphoid structures had different cell composition between stages IB and IIB tumors, where as the stage IIIB tumor was devoid of TLS. Unbiased differential gene expression analysis across three tumor types show unique cluster for stage IIIB tumor.

Multi-omics Spatial Imaging and Analysis

10x Visium CytAssist (Gene Expression) | **Lunaphore COMET (Multiplex IF)** | **Data Analysis**

Antibody Panel

Checkpoint	Structural	Stromal	T cells	Other
PD-1	E-Cadherin	α-SMA	CD3	CD11b
PD-L1	PanCK	**Cell Prolif**	CD4	Granzyme B
IDO-1	**NK Cell**	Ki67	CD8	**Endothelial**
VISTA	CD56	**Macrophage**	FOXP3	CD31
LAG-3	**B Cell**	CD68|CD206 (M2)|CD163 (M2)		**Immune**
	CD20			CD45
				HLA-DR

Non-small Cell Lung Cancer sample distribution

Stage	Pathology	TNM	Grade
I B	Adenocarcinoma	T2N0MX	G1
II B	Adenocarcinoma	T2aN1M0	G1
III B	Adenocarcinoma with micropapillary features	T3N2	G2

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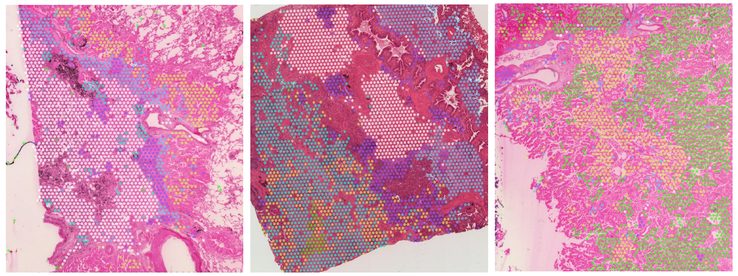
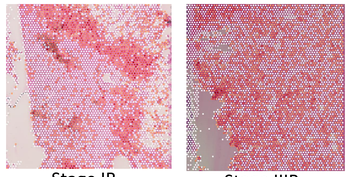
Results



Stage IB | Stage IIB | Stage IIIB

FIGURE 1 – Differential gene expression analysis across different NSCLC samples using BioTuring's SpatialX software platform: We performed an unbiased Louvain cluster analysis of 10x Genomics Visium v2 NSCLC samples to determine the spatial niches where gene expression differences were greatest between early and late stage NSCLC tissues. We were able to identify 2 clusters (Yellow and Green) that were predominantly enriched in Stage IIIB, 3 clusters (Blue, White, Turquoise) that were enriched in Stage IB and IIB, as well as a TLS enriched cluster (Purple). **Right** Stage IIIB shows higher expression of Interferon-γ related genes throughout the tissue whereas in Stage IB this is localized regions with epithelial cells



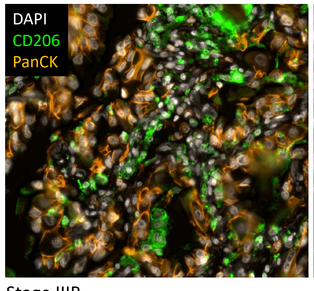
Stage IB | Stage IIIB

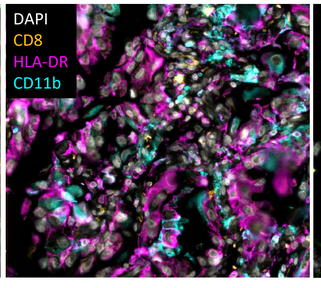
Interferon-γ related gene expression for above tumors

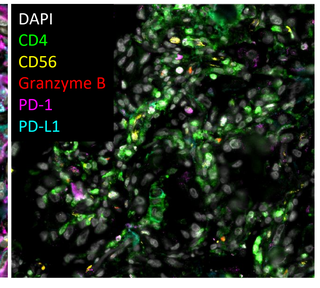
Top 10 gene expression for the unique green cluster in Stage IIIB NSCLC

Gene	Prognostic value
ANK1	Associated with tumor cell resilience
SCGB3A2	Maintain airway epithelial lineage and modulates inflammatory signaling
FMO5	Enables tumor cells to tolerate ROS and inflammatory oxidative stress. Reflects chronic immune pressure (NK/T-cell activity)
SDR16C5	Supports metabolic plasticity during stress
PCSK1N	Immune modulation
SYBU	Vesicular trafficking, potentially enabling PD-L1 expression
SPTB	Retains lineage identity often seen in immunogenic IFN-γ responsive tumor niches
SFTA2	Chronic inflammatory exposure
PGC	Tumor adaptation
REEP6	Membrane turnover and protein transport, likely facilitating PD-L1 expression

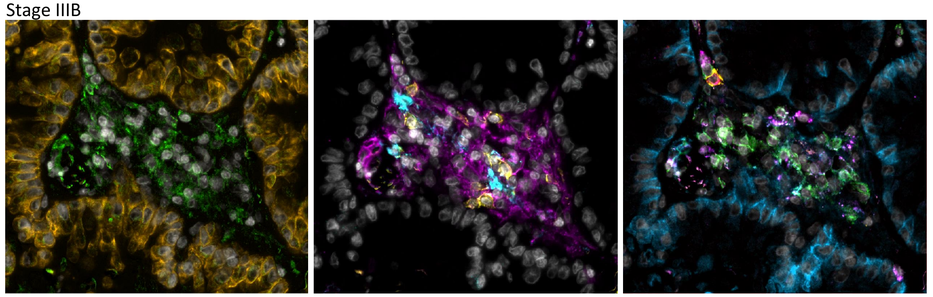
Stage IB







Stage IIIB

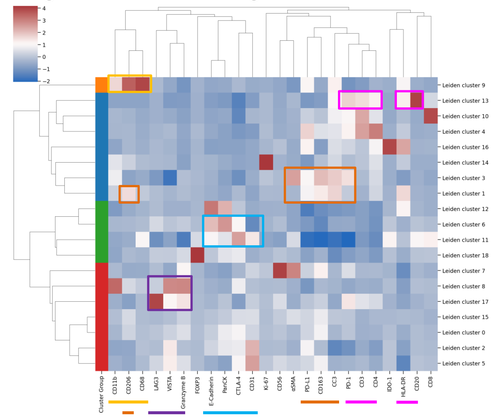


	Stage IB	Stage IIIB
Immunogenicity and Antigen presentation	High considering PanCK+ HLA-DR+ cells	Low considering PanCK+ HLA-DR- cells
TME phenotype	Likely hot	Immune evasion
CD4+ T cells	Antigen recognition and regulatory feedback to control response due to presence of CD4+FOXP3+ population	
CD8+ T cells	Present	
NK cells	Present but do not express Granzyme B	A subpopulation actively express Granzyme B
PD-L1 expression	Absent from epithelial cells	Present on epithelial cells to avoid cytotoxic response

Tumor progression score (TPS) was calculated for Stage IIIB sample on PanCK+ cells expressing PD-L1 . The score was 70%

FIGURE 2 – Tumor growth and adaptation show resistance to immune surveillance: Using HORIZON™ cell phenotyping was performed allowing visualization, Leiden cell clustering and analysis of cells expressing various markers. A striking loss of HLA-DR expression and a gain of PD-L1 expression on epithelial cells from Stage IB to IIIB was observed. Stage IIIB tumor had immune cells surveilling the tumor. There were more than one checkpoint markers expressed in these tissues.

Stage IB – Leiden clustering



Stage IIIB – Leiden clustering

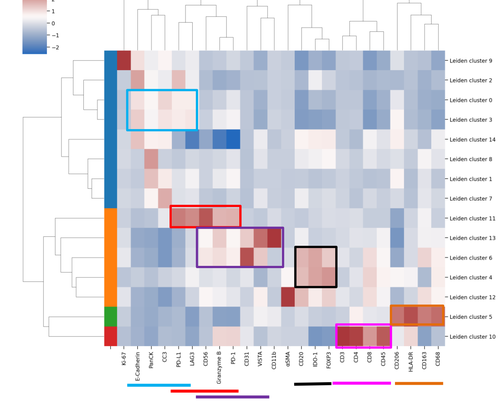


FIGURE 3 – Leiden clustering from Stage IB and IIIB tumor show more cells clustering together in advanced stage

Conclusions

- A combination of tools, identify unique cell states with clustering helping to identify closeness between different cells which vary between different NSCLC stages.
- We are currently leveraging the SpatialX platform to perform more in depth spatial gene expression analyses to study how the TME changes through NSCLC progression and any changes in gene expression pattern at different intratumoral niches and it's relationship to protein expression by overlaying Visium and mIF datasets.