B-cell Proximity Analysis in Early Stage Non-Small Cell Lung Carcinoma

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Introduction

- B-cells have been recognized as important players in the immune system's attack on cancer.[1]
- However, their exact role in this process and spatial relationship with malignant cells (MCs) are not very clear, and these concepts may be relevant to developing new therapies.[2]
- Objectives: 1) To analyze B-cells' density within and their proximity to tumor in non-small cell lung cancer. 2) To correlate the data to clinically relevant patient information and other immune cell populations.

Materials and Methods

- Twelve formalin-fixed paraffin-embedded Stage I lung adenocarcinoma tissue samples were obtained from the University of Texas MD Anderson Cancer Center archives **(Figure 1).**
- Each sample was probed with Syto13 (nucleus stain), for cytokeratin (tumor and epithelium biomarker), for CD3 (T-cell biomarker), and for CD20 (B-cell biomarker) for morphology identification **(Figure 2)**. They were also probed with nanoString panels for forty-nine immune-oncology proteins.
- The samples were processed by the GeoMX digital spatial profiler (DSP), and the images were exported to the image analysis software HALO for analysis.

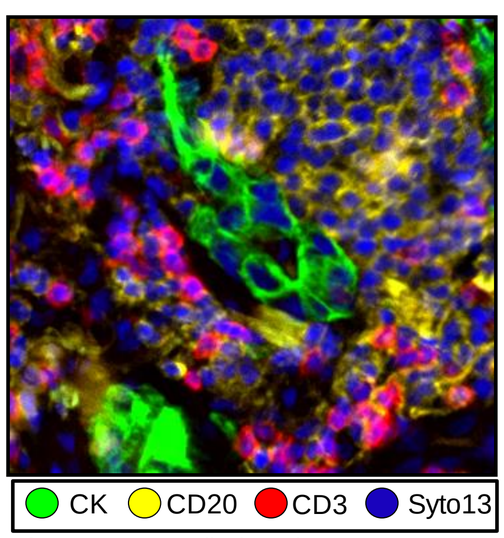

Figure 1. HALO displays the GeoMX DSP immunofluorescent morphology biomarkers simultaneously to reveal an intratumoral lymphoid structure composed of B-cells (yellow) and T-cells (red) in proximity to MCs (green).

Materials and Methods (cont.)

Characteristics	N	%
Median Age (Range)	67 (48-87)	
Sex		
Female	6	50.00%
Male	6	50.00%
Smoking Status		
Current	2	16.67%
Former	9	75.00%
Never	1	8.33%
TNM Stage		
IA	7	58.33%
IB	5	41.67%

Characteristics	N	%
Pleural Invasion		
Yes	4	33.33%
No	8	66.67%
Recurrence		
Yes	6	50.00%
No	6	50.00%
Survival		
Alive	4	33.33%
Dead	8	66.67%

Figure 1. Patient clinicopathological characteristics.

- Intratumoral B-cell density was calculated and a B-cell-tumor 20 µm spatial proximity analysis was performed **(Figure 3).**

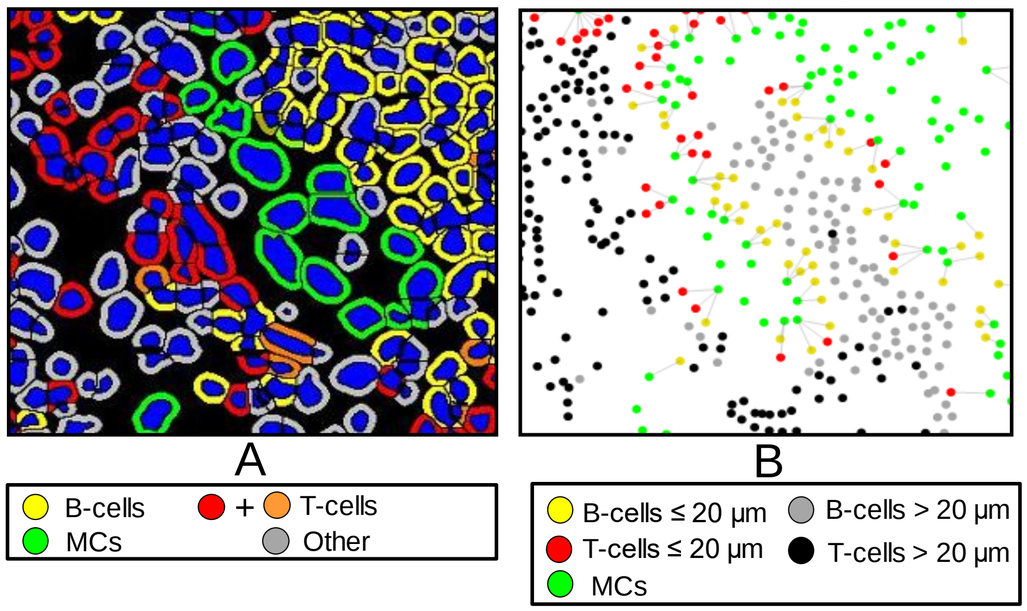

Figure 3. Spatial proximity analysis using HALO. **(A)** First, the software uses a highplex algorithm to perform cell segmentation and identifies the MCs, B-cells, and T-cells. **(B)** Then, it identifies the specific B-cells and T-cells located within 20 µm of MCs and counts them.

Results

- There was a positive correlation between intratumoral B-cell density and tumor size (r = 0.587, p = 0.045) **(Figure 4a)**, and within 20 µm of malignant cells, there was a positive correlation between the number of B-cells and the overall cell densities of tumoral macrophages and regulatory T-cells **(Figure 4b)**.

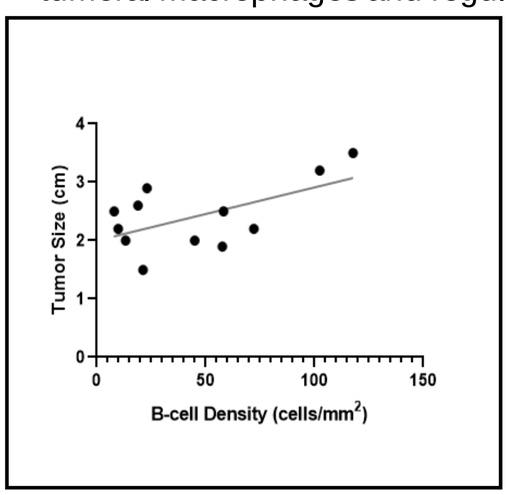

Figure 4a. Intratumoral B-cell Density was positively correlated with tumor size (r = 0.587, p = 0.045).

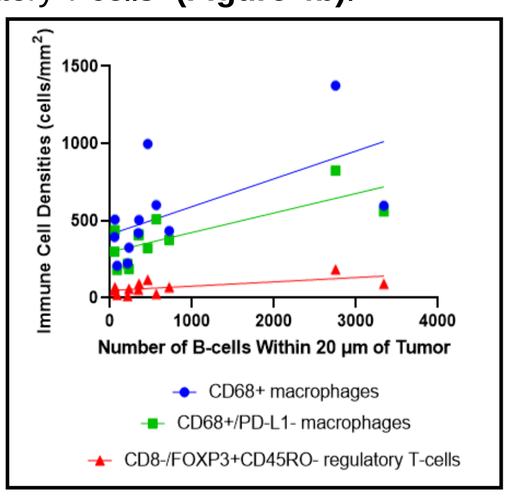

Figure 4b. B-cell count within 20µm was positively correlated with CD68+ macrophages (r = 0.5912, p = 0.0429), CD68+/PD-L1- macrophages (r = 0.7609, p = 0.0041), and CD8-/FOXP3+/CD45RO- regulatory T-cells (r = 0.6349, p = 0.0265)

Results (cont.)

- Between patients whose cancer had invaded the pleural membrane and those whose had not, there was a significant difference between the number of B cells within 20 µm of malignant cells **(Figure 5)**.

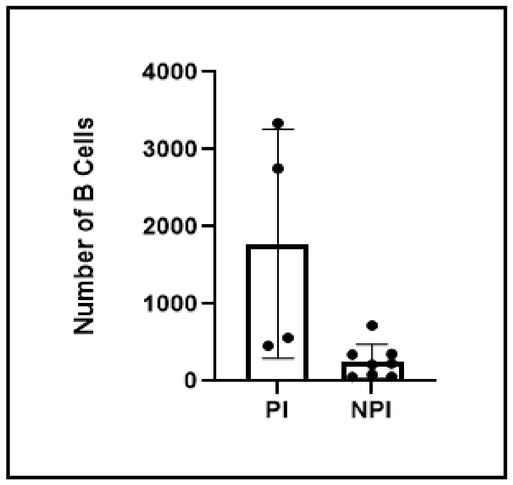

Figure 5. Patients whose cancer had invaded the pleura displayed a significantly higher number of B-cells within 20 µm of malignant cells (p = 0.0162). PI = pleural invasion. NPI = no pleural invasion.

Conclusions

- Our findings on the association between high numbers of B-cells within 20 µm of malignant cells and high densities of intratumoral macrophages and regulatory T-cells suggests that the presentation of tumor antigens is promoted even when the overall immune response is turned down.
- Future work may investigate whether the same observations, including those regarding pleural invasion and tumor size, can be validated in a larger cohort of samples.

Acknowledgements and References

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