The epithelium-stroma interface serves as a barrier to immune cell infiltration across tumor immune phenotypes in epithelial cancers

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Background

- Tumor immune phenotypes – immune-infiltrated, immune-desert, and immune-excluded, characterized by the presence and distribution of lymphocytes in the tumor bed – are associated with patient response to immune checkpoint inhibitor therapy.
- Understanding the distribution of lymphocyte density at the cancer epithelium-stroma boundary can further our understanding of immune phenotypes and provide insights into how barriers to lymphocyte entry into the cancer epithelium may impact therapeutic response to immunotherapy.

Methods

- Human tumor samples (n=99) from 5 tumor indications (colorectal, ovarian, non-small cell lung, triple negative breast, and pancreatic cancer) were classified as infiltrated, desert, or excluded by pathologist assessment.
- H&E-stained whole-slide images were further analyzed using AI-powered tumor microenvironment (TME) models developed by PathAI (Boston, MA; commercially available as PathExplore™) for tissue segmentation and cell type classification.
- Tissue segmentation was performed, and lymphocyte density was calculated in cancer epithelium, in stroma, and within varying distances (0-60 µm and 60-120 µm) from the epithelium-stroma interface (ESI) (**Figure 1**).

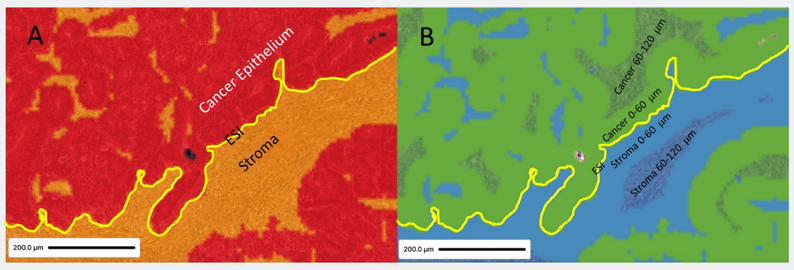

Figure 1. *CRC tumor segmentation example. Tumor sample A) segmented as epithelium (red), stroma (orange), and necrosis (black). An ESI is illustrated as a yellow line; B) segmented as ESI distance bands defined within 0-60 µm (solid) and 60-120 µm (textured) of the ESI in the cancer epithelium (green) and stroma (blue).*

- Lymphocyte density per sample, in the ESI distance bands, and the gradient of change across the ESI from the outer stroma to inner cancer epithelium were compared between tumor types and by immune phenotype.

Results

- Lymphocyte density dropped by an average of 5-fold from the stroma side to the epithelium side of the ESI in all tumor types tested (**Figure 2, Table 1**).

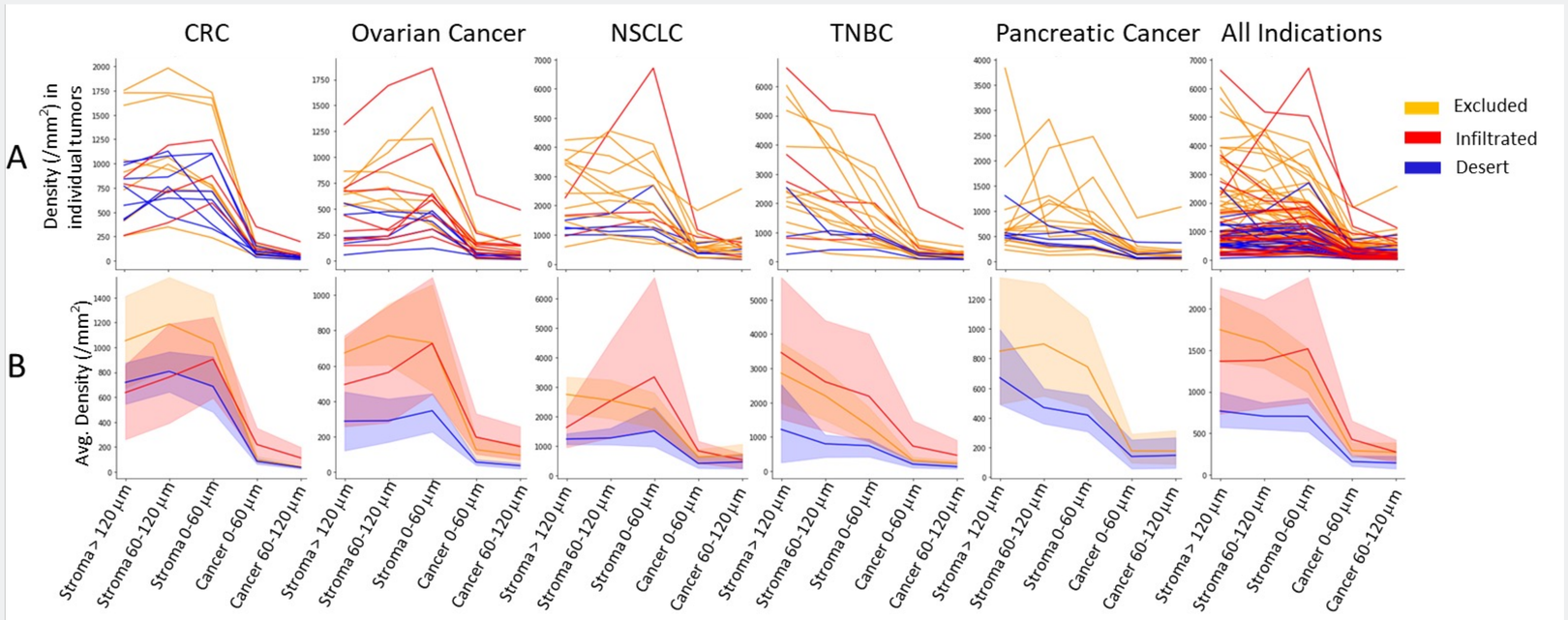

Figure 2. *Lymphocyte density in bands of cancer epithelium and stroma at different distances (0-60 µm or 60-120 µm) from the epithelium-stroma interface (ESI) by tumor type and for all indications together. A) Lymphocyte density by ESI distance band for individual tumors, colored by immune phenotype categorization. B) Averages and confidence intervals of lymphocyte density bands by immune phenotype.*

- While the observed gradient across the ESI was greatest in excluded tumors (5.9 fold change compared to baseline, range of 3.7-28.1), it was also observed in infiltrated (5.0 fold change, range 3.9-6.8) and desert tumors (5.0 fold change, range 2.7-22.3) (**Table 1**).
- The difference between excluded and infiltrated tumors was greatest in CRC, where the gradient fold-change was over four times greater in excluded than infiltrated tumors (28.1 vs 6.8) (**Table 1**).
- The most pronounced decrease in lymphocyte density occurs within 60 µm of the ESI, suggesting that barriers to lymphocyte infiltration occurred at the ESI and in the immediately adjacent stroma.

		Lymphocyte Density (cells/mm²)	FC Stroma > 120 µm	FC Stroma 60-120 µm	FC Stroma 0-60 µm	FC Cancer 0-60 µm	FC Cancer 60-120 µm (Baseline)	Difference b/t Excluded and Infiltrated
CRC (n=20)	Excluded (10)	479 ± 226	25.0	28.1	24.5	2.3	1	4.2
	Infiltrated (3)	457 ± 189	5.7	6.8	8.0	2.0	1	
	Desert (7)	366 ± 110	19.8	22.3	19.0	2.4	1	
Ovarian (n=20)	Excluded (7)	461 ± 282	7.1	8.1	7.7	1.3	1	2.1
	Infiltrated (8)	397 ± 343	3.4	3.9	5.0	1.4	1	
	Desert (5)	191 ± 88	7.9	8.0	9.5	1.6	1	
NSCLC (n=19)	Excluded (12)	1656 ± 715	4.0	3.7	3.2	0.9	1	0.8
	Infiltrated (3)	900 ± 352	3.0	4.7	6.2	1.6	1	
	Desert (4)	772 ± 342	2.7	2.7	3.2	0.9	1	
TNBC (n=20)	Excluded (13)	1427 ± 957	12.6	9.7	5.8	1.4	1	1.7
	Infiltrated (4)	2137 ± 2048	7.5	5.6	4.7	1.6	1	
	Desert (3)	762 ± 885	9.6	6.3	5.9	1.6	1	
Pancreatic (n=20)	Excluded (15)	753 ± 878	4.8	5.1	4.2	1.0	1	NA
	Desert (5)	360 ± 108	4.6	3.2	2.9	0.9	1	
All Indications (n=99)	Excluded (57)	1013 ± 861	6.5	5.9	4.6	1.1	1	1.2
	Infiltrated (18)	878 ± 1149	5.0	5.0	5.5	1.6	1	
	Desert (24)	445 ± 373	5.4	5.0	5.0	1.1	1	

Table 1. *Average lymphocyte density (cells/mm² ± standard deviation) in the tumor bed. Average fold change (FC) difference of lymphocyte density in distance bands - 0-60 µm or 60-120 µm - from the ESI on the cancer epithelium or stroma sides, as compared to baseline. Baseline is defined as the Cancer 60-120 µm ESI distance band. Values are reported by tumor type (including all indications combined) and immune phenotype. The gradient difference between excluded and infiltrated tumors is determined by the ratio of fold changes of lymphocyte density at Stroma 60-120 µm compared to baseline.*
CRC: Colorectal Cancer; NSCLC: Non-Small Cell Lung Cancer; TNBC: Triple Negative Breast Cancer

Conclusions

- Barriers to lymphocyte infiltration exist at the transition between cancer epithelium and stroma in tumors of all immune phenotypes, as assessed by H&E-based spatial features.
- While the gradient in lymphocyte density from stroma to cancer epithelium was higher in excluded tumors, a gradient was observed even in non-excluded (infiltrated and desert) tumors.
- This observation suggests that therapeutics which seek to address barriers to lymphocyte infiltration may benefit patients with all tumor immune phenotypes.

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