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Cancer-associated fibroblasts correlate with immune phenotypes in human tumors

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Background

- Cancer associated fibroblast (CAFs) are key players in immune regulation that shapes the tumor microenvironment.
- CAFs have been implicated in the exclusion of T cells from the tumor microenvironment (TME) through various mechanisms[1,2,3].
- Data related to the contribution of fibroblasts/CAFs in infiltrated, deserted, and excluded immune phenotypes in human tumor samples are lacking.

Methods

- Primary tumor resections from patients with colorectal cancer (CRC; n=20), non-small cell lung cancer (NSCLC; n=21), ovarian cancer (Ov; n=20), pancreatic cancer (Panc; n=21), triple-negative breast cancer (TNBC; n=21), leiomyosarcoma (LMS: n=20), and undifferentiated pleomorphic sarcoma (UPS; n=20) from the IMMUCAN institutional profiling program (Institut Bergonié, Bordeaux, France) were evaluated.
- Adjacent slides were stained with H&E and a multiplex immunofluorescence (mIF) panel for CD8, collagen 1 (Col1A1), and a marker of tumor cells.
- The slides were annotated by pathologists to delineate the tumor bed area and to characterize the immune phenotype of the tumors (desert, excluded, or infiltrated) using the following criteria:
  - **Desert:** characterized by a paucity/absence of CD8+ T cells;
  - **Excluded:** characterized by the presence of CD8+ T cells that do not penetrate the tumor parenchyma;
  - **Infiltrated:** characterized by the presence within the tumor parenchyma of CD8+ T cells.
- Image analysis utilizing the Akoya inForm Tissue Analysis Software was done to quantify fibroblasts (nucleated, Col1A1+ cells) and CD8+ cells in the tumor bed.

References

1. Orimo A. et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121:335–348.
2. Mariathasan S. et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature. 2018;554:544–548.
3. Jenkins L. et al. Cancer-Associated Fibroblasts Suppress CD8+ T-cell Infiltration and Confer Resistance to Immune-Checkpoint Blockade. Can Res. 2022;82:2904-2917.

Results

Relationship between CD8+ cells and fibroblasts by immune phenotype

Of 143 samples evaluated, 59 (41.2%) were classified as excluded, 38 (26.6%) as deserted, and 46 (32.2%) as infiltrated. Representative images are shown in **Figure 1**.

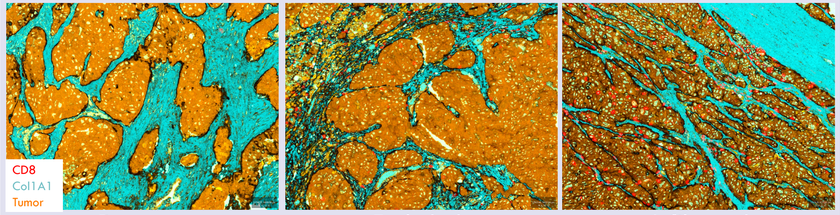

Figure 1. Representative examples of tumors categorized by immune phenotype.

CD8+ density was elevated in excluded tumors (median 257; range 40-2964 cells/mm^2) and infiltrated tumors (median 204; range 43-2976 cells/mm^2) compared to desert tumors (median 56; range 4-307 cells/mm^2) ($p<0.0001$ and $p=0.0002$, respectively) (**Figure 2**).

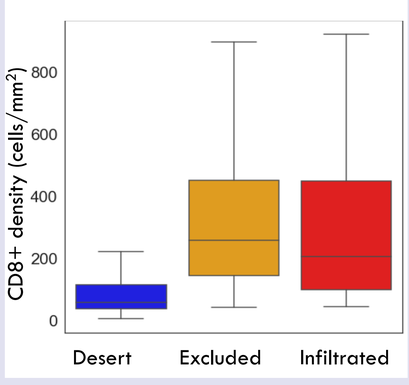

Figure 2. CD8+ cell density is elevated in excluded and infiltrated tumors relative to desert tumors.

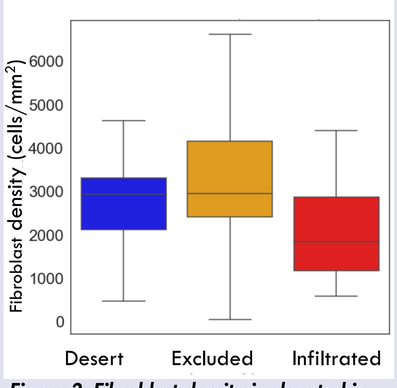

Figure 3. Fibroblast density is elevated in desert and excluded tumors relative to infiltrated tumors.

Fibroblast density was elevated in excluded tumors (median 2949; range 43-10317 cells/mm2) and desert tumors (median 2933; range 467-7987 cells/mm2) compared to infiltrated tumors (median 1836; range 581-7094 cells/mm2) ($p=0.0002$ and $p=0.0067$, respectively) (**Figure 3**).

Results (cont.)

CD8+ cell counts were directly correlated with fibroblast counts across all phenotypes (desert: r=0.66; excluded: r=0.88; and infiltrated: r=0.88; all $p<0.001$ (**Figure 4**). The correlations persisted when normalized for total cell density in the tumor bed (desert: r=0.66; excluded: r=0.77; and infiltrated: r=0.82; all $p<0.001$).

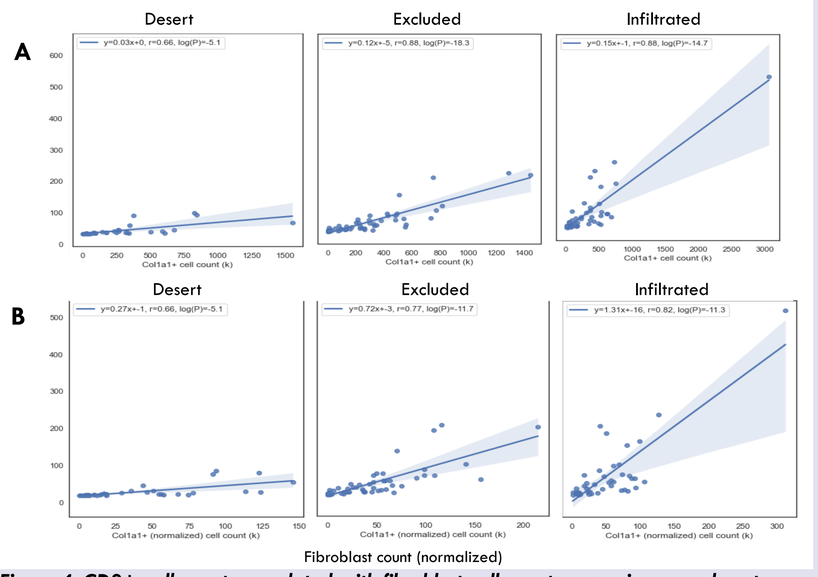

Figure 4. CD8+ cell counts correlated with fibroblast cell counts across immune phenotypes both un-normalized (A) and normalized for total cell density in the tumor bed (B).

Conclusions

- Immune excluded tumors have high levels of CD8+ lymphocytes in the TME, albeit without penetration into the tumor parenchyma. Higher CD8+ lymphocyte infiltration in the TME is associated with increased fibroblast density in all immune phenotypes. Additional work on CAF subtypes and spatial relationships within the tumor bed is needed.

Disclosures

*Dr. Sher, Mr. Gootkind, Dr. Dillon, Dr. Schürpf, and Dr. Clifton are employees of Parthenon Therapeutics. *Dr. Guegan is an employee of Explicyte Immuno-Oncology. *Dr. Italiano has research grants from AstraZeneca, Bayer, BMS, Chugai, Merck, MSD, Novartis, Pharmamar, and Roche. He serves on advisory boards. for AstraZeneca, Bayer, BMS, Chugai, Deciphera, Epizyme, Merck, MSD, Novartis, Parthenon Therapeutics, Pharmamar, and Roche.