Spatial Proteomic Profiling of VISTA and PSGL-1 Interactions Across Cancer Indications

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BACKGROUND

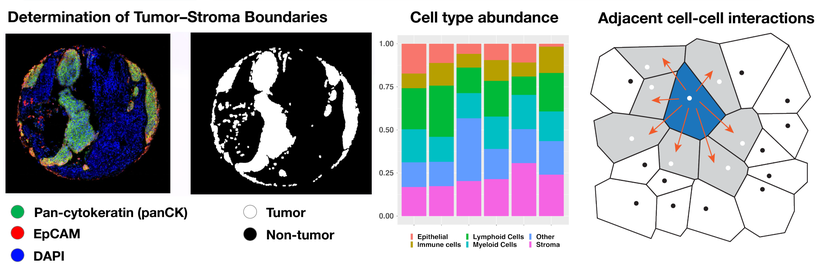
V-domain Ig suppressor of T cell activation (VISTA) is a B7 family member that regulates a spectrum of immune responses [1]. VISTA functions as an inhibitory checkpoint for T-cell activation, making it a promising target for combination cancer immunotherapy [2]. VISTA is widely expressed on immune and tumor cells but predominantly resides on tumor-infiltrating myeloid cells in multiple murine cancer models [3]. The interaction of VISTA with its ligand P-selectin glycoprotein ligand 1 (PSGL-1) is regulated by pH, and the acidic pH (~6.0) in the tumor microenvironment (TME) facilitates VISTA binding to PSGL-1 [4]. Thus, inhibiting this VISTA:PSGL-1 interaction could reduce the immune-inhibitory activity of VISTA and enhance antitumor immune responses [5,6].

STUDY AIMS

Patient selection and tumor histology are key factors in determining successful outcomes of immunotherapy. Understanding the expression of VISTA and mapping interactions between VISTA⁺ and PSGL-1⁺ immune cell populations in the TME is key to developing patient selection hypotheses that will inform clinical development strategies for VISTA-targeting drugs. We report here exploratory multiplex IHC and computational analysis of tumor tissue microarrays to profile VISTA and PSGL-1 expression on immune cells, as well as their spatial interactions, across ten cancer types.

METHODS

Multi-tumor tissue microarrays containing 24 cores from each of 10 tumor types (TriStar Technologies, Inc) were imaged by CODEX using a customized 53-plex panel of oligonucleotide-conjugated antibodies covering a variety of immune, epithelial, stromal, and functional markers. High-dimensional data were analyzed with a suite of novel computational tools developed by Enable Medicine that facilitate fast, accurate analysis at scale and in an automated fashion.



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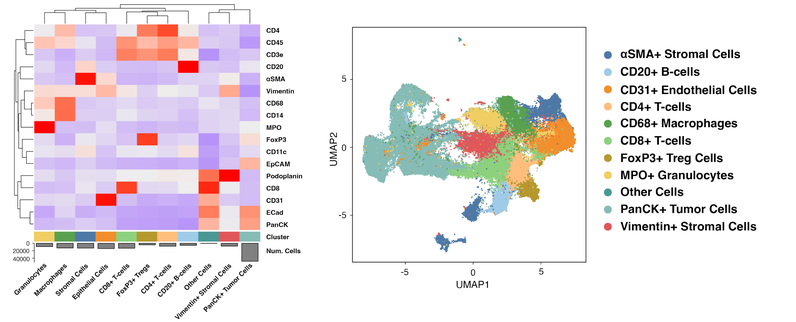

Figure 1. Cell phenotyping and clustering. Cells were segmented based on nucleus identification followed by dilation to cell membranes using DeepCell. Poorly segmented cells were filtered based on size, nuclear signal sum and signal CV. Cells were clustered based on biomarker expression similarity across each cell using unbiased Leiden clustering. Cell clusters were annotated into phenotypes and gating was used to determine biomarker positivity for the targets of interest VISTA and PSGL-1 (CD162).

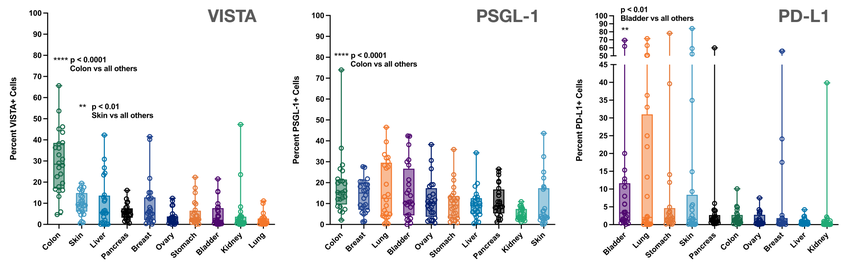

Figure 2: Expression of VISTA, PSGL-1 and PD-L1 across tumor types. Each point represents a single TMA core. Data is arranged high to low based on median. Statistical analysis was performed in R 4.1.3 using Wilcoxon rank-sum test to compare the percentages of positive populations between different tumor samples. For each tumor group, the percentages of the positive populations in samples of that group were compared with those in samples of all other tumor groups.

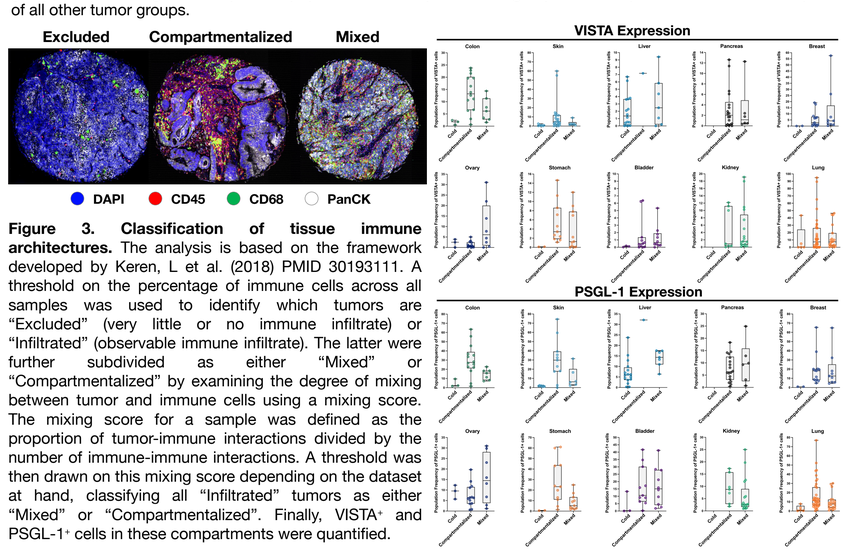

Figure 3. Classification of tissue immune architectures. The analysis is based on the framework developed by Keren, L et al. (2018) PMID 30193111. A threshold on the percentage of immune cells across all samples was used to identify which tumors are "Excluded" (very little or no immune infiltrate) or "Infiltrated" (observable immune infiltrate). The latter were further subdivided as either "Mixed" or "Compartmentalized" by examining the degree of mixing between tumor and immune cells using a mixing score. The mixing score for a sample was defined as the proportion of tumor-immune interactions divided by the number of immune-immune interactions. A threshold was then drawn on this mixing score depending on the dataset at hand, classifying all "Infiltrated" tumors as either "Mixed" or "Compartmentalized". Finally, VISTA⁺ and PSGL-1⁺ cells in these compartments were quantified.

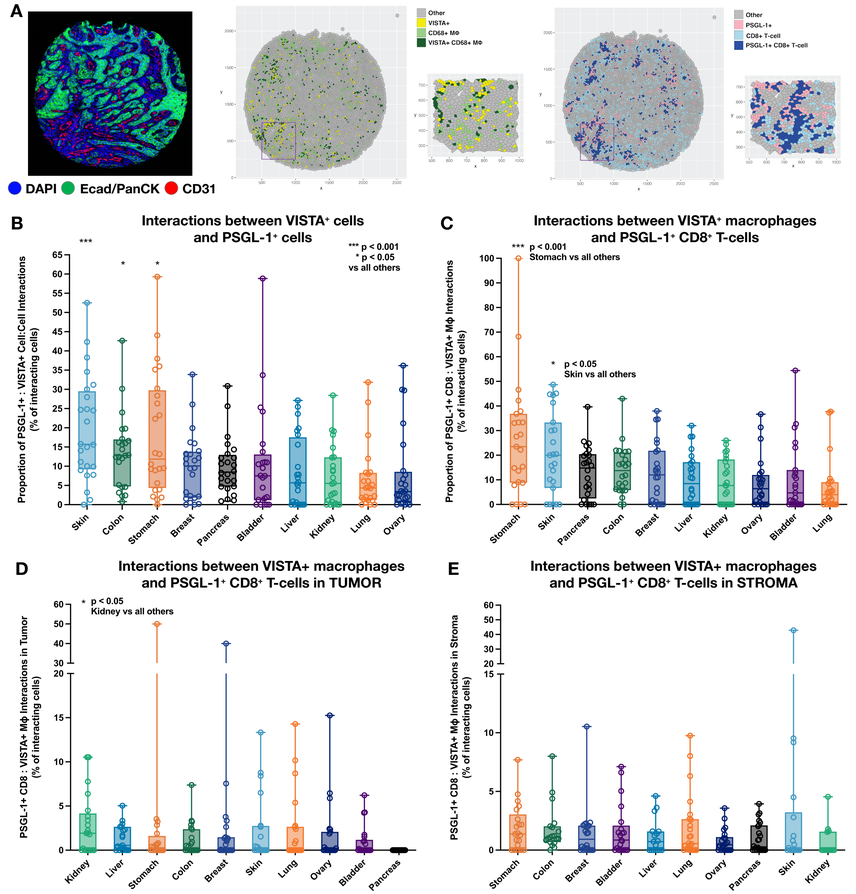

Figure 4. Quantification of VISTA⁺ and PSGL-1⁺ cell-cell interactions across tumor types. (A) Example core (stomach adenocarcinoma) with labeled nuclei (DAPI, blue), tumor (Ecad/PanCK, green) and vasculature (CD31, red) as well as corresponding Voronoi diagrams with cell types of interest labeled. (B) Proportion of VISTA⁺ cells in direct contact with PSGL-1⁺ cells across tumor types. (C) Proportion of VISTA⁺ macrophages in direct contact with PSGL-1⁺ CD8⁺ T-cells across tumor types. (D-E) Proportion of VISTA⁺ macrophage–PSGL-1⁺ CD8⁺ T-cell interactions within tumor (D) and stroma (E) across tumor types. Statistical analysis was performed as described in Figure 2.

CONCLUSIONS

- Patterns of spatial interaction between VISTA⁺ and PSGL-1⁺ cells are not captured by simple expression patterns of VISTA and PSGL-1 across tumor types
- Skin, colon and stomach cancers display the most prevalent VISTA⁺:PSGL-1⁺ cell-cell interactions, with stomach and skin cancers displaying significantly higher interactions between VISTA⁺ macrophages and PSGL-1⁺ CD8⁺ T-cells
- We hypothesize that the VISTA:PSGL-1 checkpoint may play prominent role in suppressing anti-tumor immune responses in tumors with higher levels of interacting VISTA⁺ and PSGL-1⁺ cells