Macrophages

Introduction

The complex and dynamic nature of the tumor-immune microenvironment (TME) presents challenges for identification of robust and predictive biomarkers in immuno-oncology (IO). A combination of different immune cells characterized by specific immuno-activating or inhibiting expression patterns, in addition to cytokines in the environment, present several unique niche environments not only across but within tumor samples. Gene expression profiling allows for sensitive and high-throughput analysis of genes and signatures associated with the tumor, the immune response, and the TME, allowing examination of tumor-immune cell interactions at a whole tissue high level view. Multiplex immunohistochemistry (mIHC) facilitates the ability to detect, phenotype, and quantify spatial relationships of individual cells within the tumor microenvironment. We used these approaches to generate multiple data sets from a cohort of HNSCC tumor samples.

Methods

Formalin-fixed paraffin-embedded (FFPE) specimens from HNSCC patients were cut into 5 μm sections for all technologies. nCounter PanCancer IO360™ Panel RNA was extracted from the tissues using Roche High Pure™ FFPE RNA isolation kit and analyzed for 770 genes utilizing the NanoString PanCancer IO 360 Gene Expression Panel. Transcripts were quantitated using a NanoString nCounter® and target gene counts were normalized to internal housekeeping and RNA expression from a region of interest selected on each tumor showed similar dynamic range and were selected for further analysis. The cases indicated as “Extremly Hot” and “Extremly Cold” were chosen and will herein be referred as “Hot” Tumor” and “Cold Tumor” respectively.

Results

The tumor inflammation signature (TIS) calculates a weighted average of 18 functional genes known to be associated with the PD-1/PD-L1 blockade pathway. A total of 46 tumor samples, across 4 indications were tested. The HNSCC samples showed the largest dynamic range and were selected for further analysis. The cases indicated as “Extremly Hot” and “Extremly Cold” were chosen and will herein be referred as “Hot” Tumor” and “Cold Tumor” respectively.

We used NanoString’s GeoMx Digital Spatial Profiler (DSP) to examine the “Hot” and “Cold” tumors identified by the IO360 panel. The DSP allowed us to also obtain spatially resolved analyte abundance. We showed that both protein and RNA expression from a region of interest selected on each tumor showed similar expression patterns to the nCounter data set.

Experimental Design and Workflow

Table 1. Partial List of Cell Phenotypes identified by Staining with Ultrivis’s PD-L1 Staining Kit and Using Indica Lab’s HALO Quantitative Digital Pathology.

Conclusions

The technologies described enable the investigation of the TME for use in biomarker discovery, drug discovery, and IO pathway interrogation. We used gene expression panels to screen a broad range of targets, then further investigated HNSCC specimens using the spatial analysis capabilities of DSP and mIHC. These technologies provided greater insight into the immune infiltrate profile by providing a network of cell types and co-expressing phenotypes with spatial resolution at the cell level. These complementary technologies provide useful tools in the IO biomarker toolkit.

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Labaratory Staff at Covance Companion Diagnostics – 100 Perimeter Park, Morrisville, NC

Figure 5. Ultraprobe IO-10 kit staining on HNSCC (A) 1X and (B) 20X demonstrating (C) CD8 green, (D) PD-L1 (red), (E) CD45 (red), (F) panCK (white), and nuclear counterstain (blue).

Figure 6. Ultraprobe IO PD-L1 kit staining on HNSCC (A) 1X and (B) 20X demonstrating (C) CD8 green, (D) PD-L1 (red), (E) CD45 (red), (F) panCK (white), and nuclear counterstain (blue).