Background Understanding cellular and molecular drivers of cancer cell aggression in tumors is essential to targeted therapy development and deployment to patients. Multiplex immunofluorescence and digital pathology analysis have become the standard protocol in research facilities to probe and understand these biological relationships. Antibody-based multiplex immunofluorescence can quantify hundreds of proteins in tumor cells to study how protein localization changes in pathogenic cells, while AI-based analysis methods deliver unprecedented insight into biological processes, both in fundamental cell biology and translational research. These technological advances have become important in tumor microenvironment (TME) immune profiling. Here we identified heterogeneity in exhausted T cells and used this finding to explore the association between these cells and checkpoint-blockade therapies. Identifying biomarkers that predict response to checkpoint blockade may be an important predictor of the response to immunotherapies.

Methods A tissue micro array (TMA) containing 40 tumor tissues from 27 different anatomic sites was stained using 30 antibodies from Cell Signaling Technology (CST) and imaged using the Cell DIVE Multiplex Imaging Solution from Leica Microsystems. The cores were then analyzed using HALO® image analysis platform from Indica Labs to better understand the TME.

Results We found that most tumors had an increase in expression of vimentin, alpha-SMA, survivin, and Ki67 with respect to non-tumor tissues. These markers are often upregulated in aggressive tumors and are responsible for increased cell survival, angiogenesis, and invasiveness. These tissues also expressed PD-1 and PD-L1 checkpoint signaling biomarkers at lower levels suggesting a potentially reduced response to immunotherapy. We found that some cancer types, such as esophageal cancer, squamous cell carcinoma, breast, adenocarcinoma and head-and-neck tended to have higher levels of LAG-3 and TIM-3 expression than normal tissue. Higher levels of LAG-3 and TIM-3 expression in T cells has been associated with poor prognosis and could inhibit the immune response to cancer. Anti-cancer treatment targeting TIM-3 and LAG3 could benefit patients undergoing immunotherapy. Novel combinations of these inhibitors with the current standard of care might be a promising approach to increase progression free survival.

Conclusions A combined workflow using CST antibodies, the Cell DIVE platform for highly multiplex staining and immunofluorescence imaging combined with HALO quantitative image analysis delivers a comprehensive solution for exploring the immune landscape of tumor tissue. Here, we find differential expression of markers of cancer aggression and immune cell responsiveness in a variety of cancer types. Using this critical information could help guide immunotherapy development and clinical trial stratification.

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