Analysis of the immune microenvironment across different solid tumors by combined spatial transcriptomics and proteomics

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Introduction

Resolving cell phenotypes and spatial relationships between cell populations within the tumor microenvironment will have a significant impact on our understanding of tumor progression and response to immunotherapies.

Using Miltenyi Biotec's MACSima™ Imaging System and portfolio of pre-tested antibodies, hundreds of protein markers can be visualized on a single tissue section in a non-destructive manner. Here we report on a novel combined spatial transcriptomics and proteomics approach on the MACSima Imaging System. We combined the MACSima Imaging Cyclic Staining technology (MICS) and Miltenyi Biotec's new gene expression technology RNAsky™ (for details on the RNAsky technology please refer to poster 5643) to characterize human FFPE tumor samples.

Methods

1 Spatial multiomics using the MACSima™ platform

In brief, human FFPE tissue samples were prepared for MICS (Figure 1). H&E staining was performed on an adjacent serial section for ROI selection. RNA transcript data was collected by using specifically amplified oligonucleotide detection probes conjugated to fluorochromes and using a proprietary signal removal system. Subsequently, antibody staining was performed on the same sample, using the newly established REAscreen Immuno-oncology Antibody Panel for human FFPE samples (Figure 2). Image processing, segmentation and single-cell analysis was performed in Miltenyi Biotec's MACS®iQ View software suite.

Figure 1

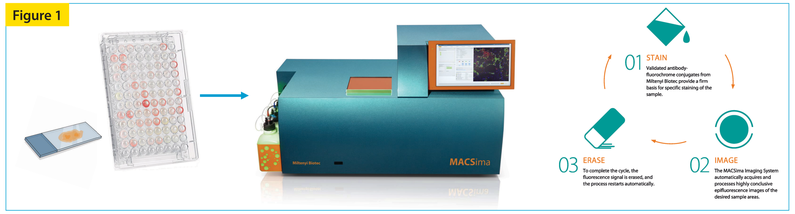


Figure 1. The MICS technology enables the simultaneous analysis of hundreds of markers on a single sample. The cyclic process comprises three main steps that are conducted repeatedly by the MACSima Imaging System in a fully automated manner:
Staining with fluorochrome-conjugated antibodies (01), image acquisition of the stained sample (02), and erasure of the fluorescent signal (3).

2 REAscreen Immuno-oncology Antibody Panel

Figure 2

Cell State	Structure	*Immune Cell Profiling*				Tumor Characterization	
Proliferation	β-Actin			CD45		Vimentin	Cytokeratin 19
PCNA	CD44	**Lymphoid**	**Myeloid**	**Immune Activation/ Function**	**Advanced Profiling**	E-Cadherin	Cytokeratin 14
Ki-67	**Stroma and Vasculature**	CD3	CD14	CD279 (PD-1)	CD45RO	EpCAM)	Cytokeratin 20
Death	CD31	CD4	CD15	CD274 (PD-L1)	CD45RA	pan-Cytokeratin	CD66e (CEA)
Cleaved PARP-1	aSMA	CD8a	CD16	CD223 (LAG-3)	CD45RB	Cytokeratin HMW	β-Catenin
Bcl-2	Podoplanin	FoxP3	CD11b	HLA-DR	CD107a	Cytokeratin 5	MART-1
	Collagen I	CD20	CD11c	CD134 (OX40)	CD107b	Cytokeratin 7	PMEL
	Calponin	CD38	CD68		CD163	Cytokeratin 8	p53
		CD79a	CD66b		CD204		
		CD138			CD209 (DC-SIGN)		
		CD56			CD1c		
		CD57			Mast Cell Tryptase		
					HLA-ABC		

Figure 2. The REAscreen Immuno-oncology Antibody Panel for human FFPE samples contains 61 markers to identify immune cells, tumor stroma including blood and lymphatic vessels, and malignant epithelial cell populations, as well as their proliferative or apoptotic state. It enables the analysis of at least 12 potential immune cell subsets within the tumor microenvironment, as well as the activation/checkpoint status of those populations.

Results

1 Spatial proteomics using MICS reveals distinct cell populations within the tumor microenvironment

FFPE tissue samples of colorectal adenocarcinoma (CRC), pancreatic ductal adenocarcinoma (PDAC), head and neck squamous cell carcinoma (HNSCC), cervical squamous cell carcinoma and melanoma were reviewed by a pathologist in order to select ROIs. 2.6 x 1.7 mm ROIs were placed in tumor regions showing high immune infiltrate. FFPE tonsil tissue was used as internal control for each MICS experiment.

Figure 3

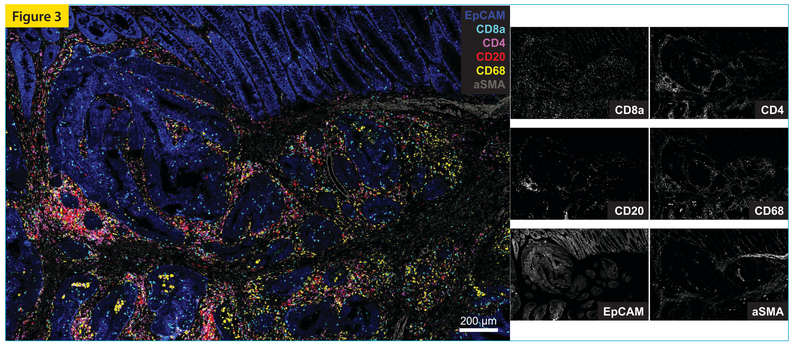


Figure 3. Whole ROI overview of a multiplex immunofluorescence staining of human colorectal adenocarcinoma tissue. The sample shows a low-grade infiltrating adenocarcinoma with colon mucosa and submucosa (upper right) and a prominent lymphocyte infiltrate. A tertiary lymphoid structure is visible towards the left of the ROI. Biomarkers as indicated; single stains of each marker are displayed to the right.

Figure 4

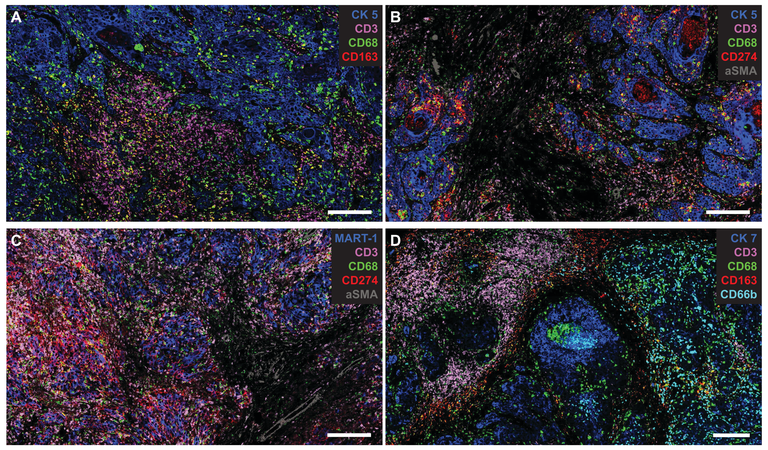


Figure 4. Magnified overviews of multiplex immunofluorescence stainings of human cancer tissues: A) cervical squamous cell carcinoma, B) head and neck squamous cell carcinoma, C) melanoma, D) pancreatic ductal adenocarcinoma. The different tissues show distinct distribution of several immune cell populations within the tumor microenvironment.
Biomarkers as indicated, overlap of red and green staining results in yellow color. Scale bars: 200 µm.

Figure 5

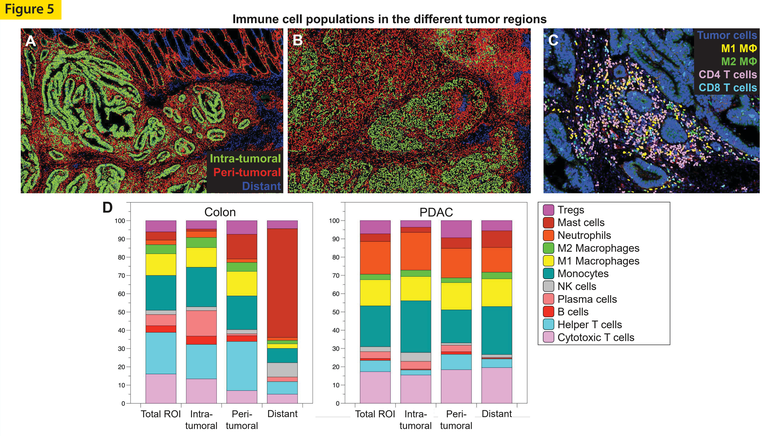
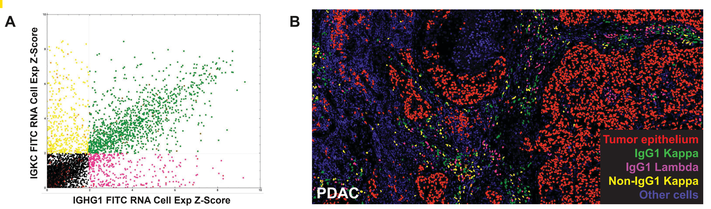


Figure 5. Immune cell populations were identified by cell gating, and distance maps were created. The intra-tumoral region was defined by the tumor cells and a surrounding distance of 5 µm, the peri-tumoral region included the adjacent 300 µm. Distance maps of the human CRC (A) and PDAC (B) samples. C) Magnification of the CRC sample, showing the identified immune cell populations. D) Percent of the indicated immune populations per total number of immune cells within the respective tumor region. Cell populations were gated as follows: Tregs - $CD4^+$ $FoxP3^+$, mast cells - mast cell $tryptase^+$, neutrophils - $CD11b^-$ $CD66b^+$, M1 macrophages - $CD14^+$ $CD68^+$ $CD163^-$, M2 macrophages - $CD14^+$ $CD68^+$ $CD163^+$, Monocytes - $CD11b^+$, NK cells - $CD56^+$, Plasma cells - $CD79a^+$, B cells - $CD20^+$, helper T cells - $CD4^+$, cytotoxic T cells - $CD8^+$.

2 The combination of proteomics and transcriptomics allows for unprecedented insight into cell phenotypes in a spatial context

Within a PDAC sample, cells were segmented and plasma cells identified by the marker CD79a. Subsequently, fluorescence signals for IGKC and IGHG1, generated by the RNAsky technology, were gated (Figure 6A) to determine plasma cell relationship based on immunoglobulin kappa or lambda light chain usage. 45% percent of IgG1-expressing plasma cells expressed a lambda light chain, 55% a kappa light chain, indicating a normal kappa/lambda ratio of the tumor infiltrating plasma cells. Distribution of plasma cell clones within the tumor microenvironment is shown in Figure 6B.

Figure 6



Conclusion

With the introduction of the RNAsky™ technology, the MACSima™ has evolved to a true multiomics platform enabling protein and RNA detection on a single section. The shown characterization dataset demonstrates the ability of our platform to perform standardized in-depth phenotyping of sample cohorts. This will enable discovery and further development of predictive and prognostic biomarkers critical to patient stratification, clinical management and response to immunotherapy.

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