Spatially resolved multiplex immunofluorescence profiling of antibody-drug conjugate targets in bladder cancer using an AI-powered end-to-end workflow

Christoph Kuppe[1], Markus Eckstein[2], Samaneh Samiei[1], Katharina Dornblut[3], Niklas Klümper[4], Fabian Schneider[5], Moritz Widmaier[3], Florian Leiss[3]

[1]University Hospital RWTH Aachen, Aachen, Germany, [2]FAU Erlangen-Nürnberg, Nürnberg, Germany, [3]ZEISS Microscopy GmbH Jena, Germany, [4] University Hospital Bonn, Bonn, Germany, [5]Mindpeak GmbH, Hamburg, Germany

Abstract

Bladder cancer is one of the most common malignancies, with significant mobidity and mortality rates. However, insight about the location and presence of different ADC target molecules is needed to guide treatment decisions. Multiplex immunofluorescent (mIF) of ADC targets offers the opportunity to investigate multiple biomarkers and their spatial distribution at the same time, enabling detailed spatial analysis of ADC targets within a tissue section.

Spatial multi-omics and single cell technologies applicable to patient tissues like formalin fixed paraffin embedded tissue (FFPE) open the possibility to unlock these spatial molecular profiles which ultimately could be used to identify companion diagnostic biomarkers and providing valuable insights into tumor microenvironment heterogeneity to optimize treatment efficacy and minimize off-target effects which we persue in DECODE-ADC.

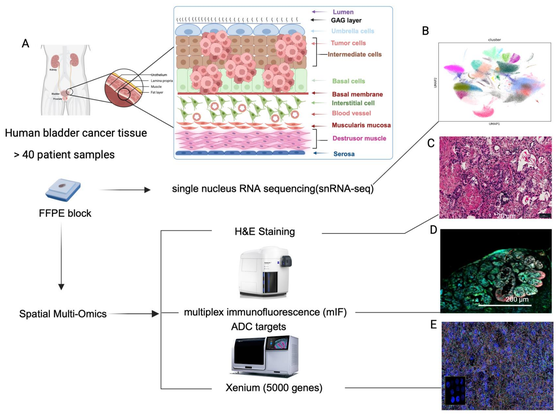

Fig. 1. Experimental workflow. **A**. Formalin-fixed paraffin embedded (FFPE) tissue samples of over 40 patient samples are used for a spatial-multiomics map of metastatic urothelial cancer tissue samples. **B**. Single-cell RNA sequencing data was generated using nuclei isolated from FFPE blocks **C**. H&E stainings **D**. multiplex immunofluorescence (mIF) staining of ADC targets. **E**. Spatial transcriptomic profiling using Xenium targeting over 5.000 transcripts.

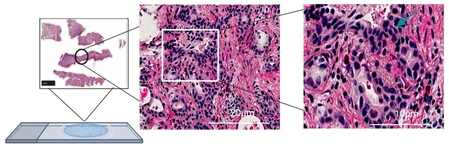

Fig. 2. Expert pathologist annotations of tumor-microenvironment based on histological analysis of the primary bladder tumor in mUC patients; cell nuclei (blue-purple) in blue arrow, other cell bodies and extracellular matrix (pink), tumor cells (blue arrow).

Methods

The DECODE-ADC project integrates single-cell and spatial multi-omics data from FFPE tissues of EV-treated patients, encompassing matched pairs of primary tumors and metastatic biopsy from a cohort of >40 patients. Single-nucleus RNA sequencing and high-resolution spatial transcriptomics were used to profile various ADC targets with additional markers of the tumor microenvironment and drug resistance (over 5000 gene panels). We utilized a novel mIF reagent system to analyze FFPE bladder cancer samples. Slides were imaged using ZEISS Axioscan 7 spatial biology system and SlideStream automation for high-throughput, standardized acquisition. Image analysis was performed using Mindpeak PhenoScout integrated into the automated workflow, which employs pre-trained AI models for tissue segmentation, single cell detection, biomarker positivity and phenotype classification based on multichannel signal integration.

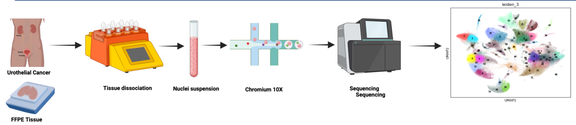

Fig. 3. Single nuclei RNA sequencing workflow steps: starting material derived from FFPE blocks, dissociation using enzymatic and mechanical digestion (Milteny) and processing of nuclei using 10XGenomics Flex assay.

Results

Using spatial single-cell multiomics, we identified novel spatial domains regulating ADC target expression and delineated tumor microenvironment-specific signals. We established an mIF assay to investigate different ADC targets in bladder cancer samples. This information, in combination with AI-based analysis, was used to generate an ADC sensitivity profile for each patient.

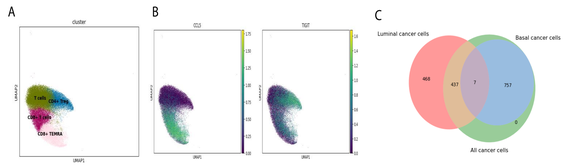

Fig. 4. Uniform manifold approximation and projection (UMAP) of T cells. **A.** Subtypes of T cells expression in single-cell RNA sequencing. **B.** CCL5 and TIGIT are highly expressed in CD8+ T cells and in CD4+ Treg cells. **C.** Differentiation of luminal cancer cells, basal cancer cells and all cancer cells using Venn diagram.

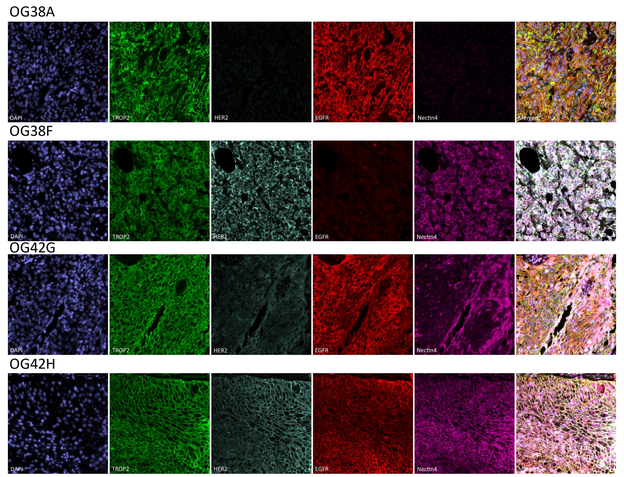

Fig. 5. Multiplex immunofluorescence (mIF) of ADC targets using Axioscan (Zeiss) and Revolune reagents. Overview mIF image of a human primary bladder cancer tissue in 4 patients. IF DAPI (nuclei) and TROP2, HER2, EGFR and NECTIN4.

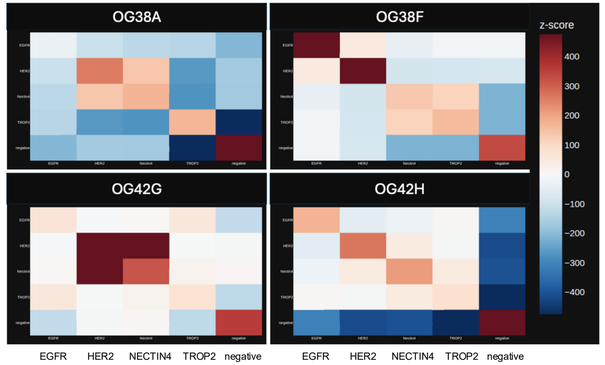

Fig. 6. 30 µm Neighbor Frequency Analysis for 4 Cases: OG38A, OG38F, OG42G, OG42H within the Tumor polygon analysis. Tumor polygon was generated with TROP2 expression in these cases. dark red: 100% likelihood, dark blue no neighbors within 30µm of each tumor cell. PhenoScout AI: DAPI was used for Cell center detection. PhenoScout AI foundation model: mIF membrane was used to identify positive tumor cells for each biomarker: EGFR, HER2, TROP2 and Nectin-4. Radius for neighbor frequency analysis: 30µm around each cell within the tumor polygon region.

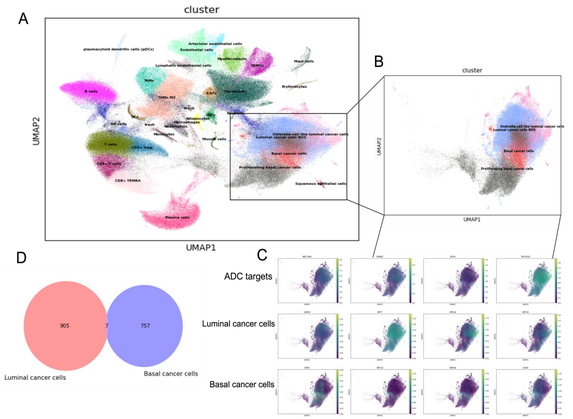

Fig. 7. Uniform manifold approximation and projection (UMAP) of single-cell RNA sequencing cell cluster annotation. **A.** UMAP. **B.** luminal cancer cells and basal cancer cells. **C**. ADC target markers are highly expressed in cancer cells cluster (NECTIN4, ERBB2, EGFR and TACSTD2), luminal cancer cell markers (GATA3, KRT7, UPK1A and KRT20) and basal cancer cell markers (KRT5, KRT14, KRT6A and CD44) also highly expressed in cancer cells. **D.** Venn diagram shows the difference between the luminal cancer cells and basal cancer cells.

Conclusions & Outlook

DECODE-ADC provides unprecedented insights into the spatial dynamics of ADC targets within the tumor microenvironment of mUC. By integrating advanced molecular profiling with ML-driven predictions, this study advances our understanding of ADC target expression and establishes a pathway for rational biomarker selection. Spatially resolved miF analysis of bladder cancer revealed clinically relevant biomarker signatures, highlighting its potential for patient stratefication. The integration of automated imaging and AI-driven analysis ensures robust, reproducible spatial profiling, accelerating the translation of multiplex tissue imaging into precision oncology and personalized treatment approaches. Our initial findings propose a foundation for precise ADC biomarker development and stratification strategies to improve treatment ADC responses.

Corresponding authors contact

Prof. Dr. Dr. Christoph Kuppe
Universitätsklinikum RWTH Aachen
E-mail: ckuppe@ukaachen.de
website: https://www.kuppelab.org
phone: +491775988719

PD Dr. med. Markus Eckstein
Universitätsklinikum Erlangen
email: markus.eckstein@uk-erlangen.de
website:
phone:

Collaborators

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