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Spatial Transcriptomics – a valuable tool to visualize compound effect in precision cut cancer tissue slices (PCCTS)

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INTRODUCTION

The recent advances in immunotherapies, such as immune checkpoint modulators, bispecific antibodies, and adoptive T-cell transfer, opens new opportunities for the treatment of cancer. Having this broad spectrum of new therapeutic agents available, the demand for predictive and robust preclinical models to minimize translational failures in immuno-oncology is increasing. Indivumed has successfully implemented a model of Precision Cut Cancer Tissue Slices (PCCTS) derived from viable human tumor tissue for different applications such as chemotherapeutic agents, small molecules and antibodies.

In this study, we investigated the effects of OKT3® (Muromonab), a therapeutic antibody against CD3, on PCCTS from a patient diagnosed with non-small cell lung cancer (NSCLC) in respect of gene expression changes using the 10x Genomics Spatial Transcriptomics technology.

METHODS

Samples: Vital tumor tissue from a patient diagnosed with non-small cell lung cancer (NSCLC) was collected immediately after resection according to Indivumed's standard operating protocols. Informed consent was obtained from that patient.

Preparation of Precision Cut Cancer Tissue Slices (PCCTS): Vital tumor tissue from one NSCLC patient was used as starting material for the preparation of PCCTS. Therefore, fresh tumor tissue was cut into 500 µm slices using a Krumdieck™ tissue slicer (TSE Systems).

Cultivation and drug treatment: PCCTS were cultivated in a supplemented RPMI 1640 tissue culture medium in 24 well plates. For drug treatment PCCTS were pre-cultured for one hour. Subsequently, PCCTS were incubated for 24 h with and without 10 µg/ml OKT3®, (Muromonab), a therapeutic antibody against CD3. For each condition three PCCTS were treated. After 24 h, slices were individually frozen and stored in liquid nitrogen until further processing.

10x Genomics Visium Spatial Gene Expression Workflow: One frozen PCCTS per condition was sectioned in a cryostat and one 10 µm section per PCCTS was mounted on a Visium Spatial Gene Expression Slide containing spatially barcoded capture probes binding mRNAs of a tissue section. Afterwards, the PCCTS sections were first H&E stained and scanned to visualize the histological tissue structure. Subsequently followed by the Visium Spatial Transcriptomics Library Preparation including tissue permeabilization of 20 min, cDNA synthesis, cDNA amplification and library construction. The final libraries were sequenced at a concentration of 300 pM on a NovaSeq™6000 using a SP v1.5 flowcell (Illumina) to reach a minimum sequencing depth of 50.000 read pairs per tissue covered spot by a third-party provider. Sequencing data containing the specific spatially sequencing barcodes were analyzed using the Space Ranger analysis pipeline and the Loupe Browser to assign the gene expression data to the corresponding histological positions in the tissue section (Figure 1).

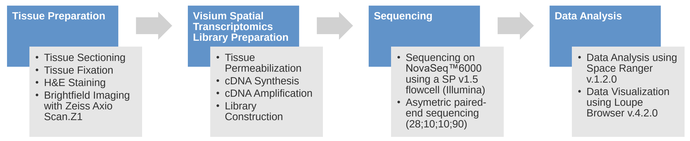

Figure 1: Schematic illustration of the Visium Spatial Gene Expression Workflow (10x Genomics).

Precision cut cancer tissue slices platform & experimental design

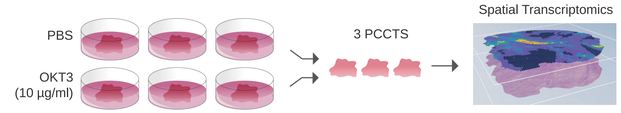

Figure 2: PCCTS platform and experimental setup. The Precision Cut Cancer Tissue Slice (PCCTS) platform was used to cut fresh NSCLC tissue into 500 µm slices. Three PCCTS per conditions were incubated for 24 h with or without 10 µg/ml OKT3®. Following incubation, PCCTS were frozen and stored in liquid nitrogen. One PCCTS per condition was used for the 10x Genomics Visium Spatial Gene Expression Workflow.

RESULTS – Spatial Gene Expression in OKT3® treated and untreated PCCTS

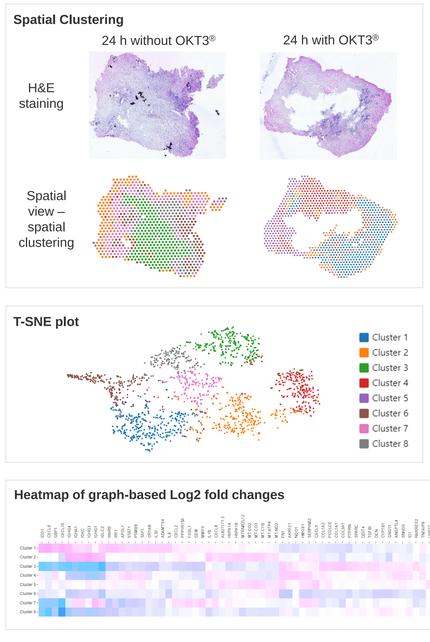

Figure 3: H&E stain, spatial view, t-SNE plot and Heatmap (graph-based cluster analysis) of NSCLC tumor tissue PCCTS incubated 24 h without and with 10 µg/ml OKT3®. Graph-based cluster analysis based on a "spacerange aggr" analysis combining both samples into a single datasheet. Spatial gene expression technology identified 8 clusters within both samples. Histopathological and spatial cluster evaluation revealed that gene expression cluster 6 (brown) reflects the tumor cell areas within both samples, the cluster 1 reflects the microenvironment of the 24 h OKT3® treated PCCTS (blue) and the cluster 3 and cluster 8 reflect the microenvironment of the OKT3® untreated PCCTS (green and grey). T-SNE and Heatmap view showed clear cluster formation and differentially regulated genes between clusters and treatment.

RESULTS – Spatial clustering revealed OKT3® induced gene expression changes in tumor microenvironment

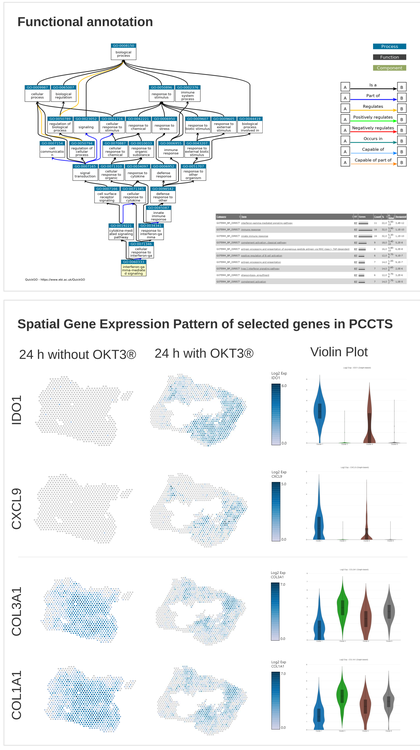

Figure 4: Pathway analysis. Functional annotation was performed by up-loading the 50 most up-regulated genes of cluster 1 (microenvironment of OKT3® treated PCCTS) into the DAVID Bioinformatics Database v6.8 (ranking of molecular pathways by selected genes).
Gene Ontology analysis considering biological processes (GOTERM_BP_DIRECT) clearly indicates that immune response processes especially the interferon-gamma signaling pathway are induced in the microenvironment of OKT3® treated PCCTS. Spatial pattern of IDO1 and CXCL9 demonstrated strongly increased gene expression within OKT3® treated compared to untreated PCCTS predominantly located in the microenvironment and tumor cell cluster (cluster 1 and 6).
Analysis of the 50 most up-regulated genes in cluster 3 (microenvironment of OKT3® untreated PCCTS) revealed that these genes contribute to extracellular matrix pathways indicating cellular processes typically active in cells of the microenvironment (data not shown). Examination of COL3A1 and COL1A1 gene expression showed expression in all analyzed clusters with the highest expression in the microenvironment of untreated PCCTS (cluster 3 and 8).

CONCLUSION AND SUMMARY

- 10x Genomics spatial gene expression analysis enabled the identification of cellular subpopulations in the spatial context before and after treatment with OKT3®
- Spatial gene expression data showed significant differences between untreated and OKT3® treated tissue slices especially in the microenvironment that encompasses inflammatory cells, extracellular matrix, and stromal cells interacting with tumor cells for cancer growth and progression
- Pathway analysis showed a clear immune stimulatory effect of OKT3® in NSCLC PCCTS
- PCCTS platform has been shown to be most valuable for the understanding of compound effects