Poster 1711

Immunoprofiling of Precision Cut Cancer Tissue Slices (PCCTS) as a Tool for Cancer Immunotherapy

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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® on cancer tissue slices especially in respect of gene expression changes and cytokine release.

Methods

Samples: Vital tumor tissue from three colorectal cancer (CRC) patients were collected immediately after resection according to Indivumed's standard operating protocols. Informed consent was obtained from all patients.

Preparation of Precision Cut Cancer Tissue Slices (PCCTS): Vital tumor tissue from CRC patients were used as starting material for the preparation of PCCTS. Therefore, fresh tumor tissues were cut into 900 µm slices using a Krumdieck™ tissue slicer (TSE Systems).

Cultivation and drug treatment: PCCTC 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 4h and 24h with and without OKT3®, (Muromonab), a therapeutic antibody against CD3. For each condition three PCCTS were treated. After defined time points, slices were frozen, and the supernatants were collected. In addition, a second set of slices was formalin fixed and paraffin embedded for analysis of T-cells.

RNA isolation and sequencing: RNA isolated from frozen slices was used for library preparation with the TruSeq Stranded mRNA Library Preparation Kit followed by sequencing on a NextSeq 500 device from Illumina. The three PCCTS for each condition were analysed separately.

Meso Scale Discovery (MSD): The analysis of cytokines in the supernatants of tissue cultures was performed using the validated ten-plex proinflammatory panel from MSD. Supernatants for each condition were pooled and analysed.

Immunohistochemical (IHC) Staining: Anti-CD3 was implemented on the DISCOVERY XT/Benchmark Ultra staining platform (Ventana). Image analysis was conducted using Axio Scan.Z1, Zeiss. The three PCCTS for each condition were pooled and embedded in one FFPE block.

Figure 1: Schematic illustration of the technical workflow of the "Precision Cut Cancer Tissue Slice" platform.

Experimental Setup

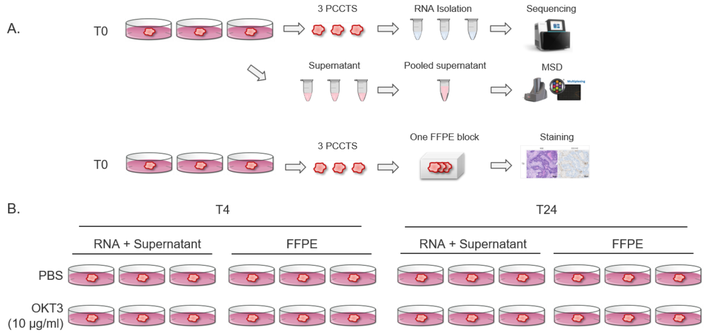

Figure 2: Schematic illustration of experimental setup for **A.** timepoint T0 after pre-cultivation. PCCTS were fresh frozen for RNA sequencing and supernatants were collected for analysis of cytokine release. A second set of PCCTS were formalin fixed and paraffin embedded for subsequent IHC analysis. **B.** Experimental setup at 4h (T4) and 24h (T24). PCCTS and supernatants were collected for RNA sequencing, IHC and MSD analysis.

Results (RNA Sequencing)

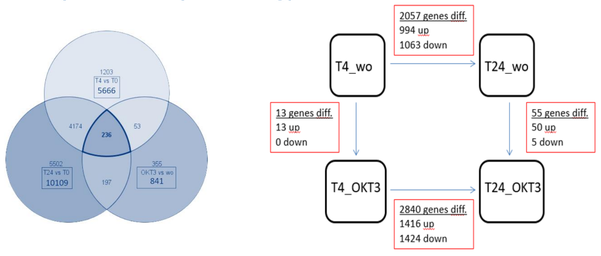

Figure 3: A. Venn Diagram: the vast majority of differentially expressed genes can be detected between T24 and T0, followed by T4 and T0. OKT3 dependency yields the smallest number of differentially expressed genes with a significant overlap of 28% to time dependent differentially expressed genes which points to at least partially overlapping effects. **B. Differentially expressed genes:** Despite the high background of gene expression changes caused by cultivation, there are additional differentially expressed genes detectable after OKT3 treatment although to a smaller extend. Induction of IFN-γ signalling, IL-12 signalling and other immune related functions start around 4h after treatment and continue up to 24h.

Table 1: Main pathways influenced by OKT3 treatment

- IL-12 mediated signaling events
- IL-23 mediated signaling events
- IL-27 mediated signaling events
- IFN-γ pathways
- Th17 cell differentiation
- IL-12 signaling mediated by STAT4

Results (CD3 Expression)

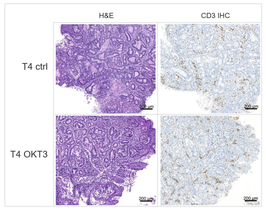

Figure 4: H&E and anti-CD3 IHC of human FFPE colorectal cancer (CRC) tissue. A strong membranous anti-CD3 staining of immune cells was detected.

Table 2: Evaluation of anti-CD3 IHC of CRC tissue samples.

Tissue type	Tumor content [%]	Infiltration of CD3-positive cells					
		Tumor region					
		HPF1	HPF2	HPF3	HPF4	HPF5	Average
CRC	30	165	183	98	208	252	186
CRC	25	143	127	232	272	243	203
CRC	40	64	110	156	241	76	129

The number of CD3-positive immune cells per High-Power Field (HPF) were quantified in the tumor region and in the peritumoral stroma. Five HPFs were evaluated per sample and region, and the average numbers of CD3-positive immune cells per HPF were calculated. CD3-positive cells were detected in all CRC tissue samples, showing averages from 129 to 203 CD3-positive cells per HPF.

Results (Cytokine Release)

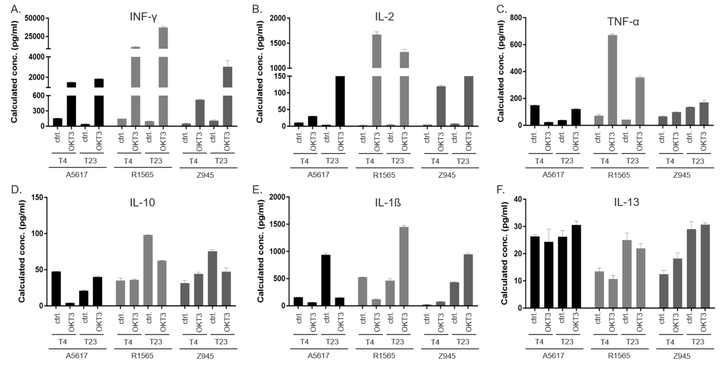

Figure 5: Cytokine secretion of untreated and OKT3 treated PCCTS from three CRC patients. PCCTS were treated with 10 µg/ml OKT3 for 4 and 24 hours. Cytokine secretion was analyzed in supernatants of tissue cultures using the validated ten-plex proinflammatory panel from MSD. Shown is the mean value with standard deviation of cytokines in pg/ml compared to the untreated control.

Conclusion and Summary

- OKT3 treatment induced cytokine secretion into the supernatant. Especially high levels of INF-γ, IL-2, and TNF-α were detectable.
- RNA preparation from Indivumed's PCCTS platform is suitable for subsequent RNA-Sequencing.
- OKT3 immune stimulatory effects were also measurable by RNA sequencing.
- The model of PCCTS is suitable for pre-clinical evaluation of immunomodulatory compounds.