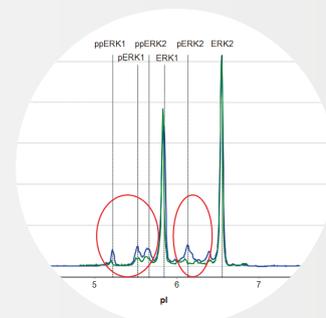indivumed

Drug profiling using human precision cut cancer tissue slices:

Treatment of tissue slices with a monoclonal anti-EGFR antibody and analysis of functional effects

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In order to improve **anti-cancer drug development** and **personalized therapies,** novel and highly predictive *in vitro* drug testing systems are needed to examine the characteristics of each individual tumor. Indivumed has already developed a preclinical drug testing platform based on precision cut cancer tissue slices that is suitable for the investigation of anti-cancer drug effects (chemotherapeutics) in the natural tumor microenvironment.

We further advanced this model for testing the application of targeted drugs such as therapeutic antibodies. In the present study we examined the functionality of a monoclonal anti-EGFR antibody using our drug testing platform. To do so, colorectal cancer tissue samples freshly obtained from surgical specimens of several patients were treated with an anti-EGFR antibody for up to 48 hours. The EGFR expression status of the tissue samples was analyzed by immunohistochemical (IHC) staining. Tissue proliferation was determined by Ki67 levels. As readout of antibody-related therapeutic effects, the phosphorylation levels of selected key proteins from two EGFR-related downstream pathways, i.e., the MAPK-pathway and Akt-pathway, were evaluated by the Meso Scale Discovery (MSD®) multiplex technology and IHC staining.

For a more detailed view of signaling pathways, the NanoPro™ 1000 technology was integrated into our pathway analysis approach, enabling the identification of distinct isoform phosphorylations.

Keywords:

- Preclinical Drug Testing
- Human precision cut cancer tissue slices
- Tumor Microenvironment
- Anti-CancerTargeted Therapy

Material and Methods

Tumor tissue pieces of colorectal cancer (CRC) patients were collected immediately after resection and subsequently sliced into 400 µm slices using a tissue slicer. Tissue slices were then transferred to 24-well plates and cultivated for up to 48 hours (see Figure 1). CRC tissue slices were pre-cultured for one hour before the anti-EGFR antibody was added at three different concentrations and incubated up to 48 hours. Samples treated with a control IgG pool at a single concentration served as additional control.

Proteins from signaling pathways were analyzed by Meso Scale Discovery (MSD®) assays (Meso Scale Discovery USA), immuno-histochemistry, and NanoPro™ 1000 technology. For quantification of total and/or phosphorylated Akt, mTOR, p70S6K and ERK1/2 (MAPK) tissue lysates were subjected to the respective 96-well MSD® plate in triplicates, and MSD® assays were performed according to the manufacturer's instructions. Further analysis of ERK1/2 (MAPK) was conducted using the NanoPro™ 1000 technology platform. This technology enables the identification of multiple isoform phosphorylations according to their isoelectric point. Protein lysates were separated on a nested Premix G2 5-8 gradient against the pI standard ladder 3 and immobilized for 70 seconds.

For immunohistochemistry, tumor tissue slices of each case and condition were formalin fixed, paraffin embedded and cut into slices. Automated IHC was performed for EGFR, Ki67, and pERK1/2 using the Benchmark® Ultra (Roche Diagnostics Deutschland GmbH). Stained sections were examined under microscopy.
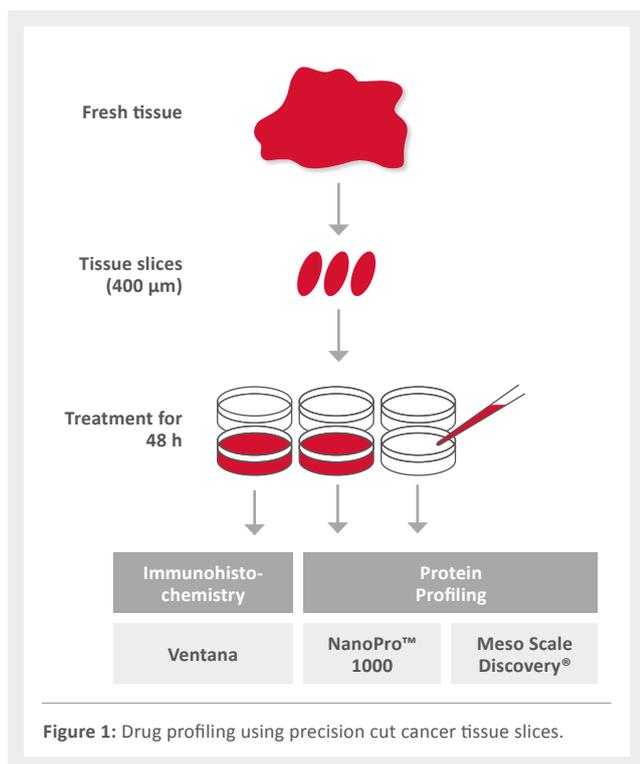

Figure 1: Drug profiling using precision cut cancer tissue slices.

Results

In order to determine and verify drug effects of a therapeutic antibody in precision cut cancer tissue slices, tumor tissue slices from colorectal cancer patients have been treated with an anti-EGFR antibody in three different concentrations for 24 and 48 hours. The effectiveness of the anti-EGFR antibody is indicated by the reduction of expression and phosphorylation levels of signaling proteins from two EGFR downstream pathways, the Akt pathway and the MAPK pathway. The EGFR receptor status in different tumor tissues was determined by immunohistochemical staining (IHC). IHC staining revealed that expression levels ranged from moderate to strong (Figure 2). The proliferation marker Ki67 demonstrated that tissue remained viable and in a proliferative stage.
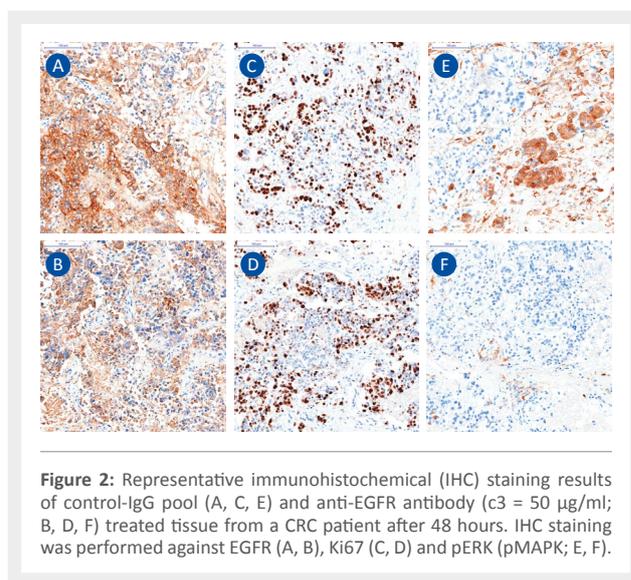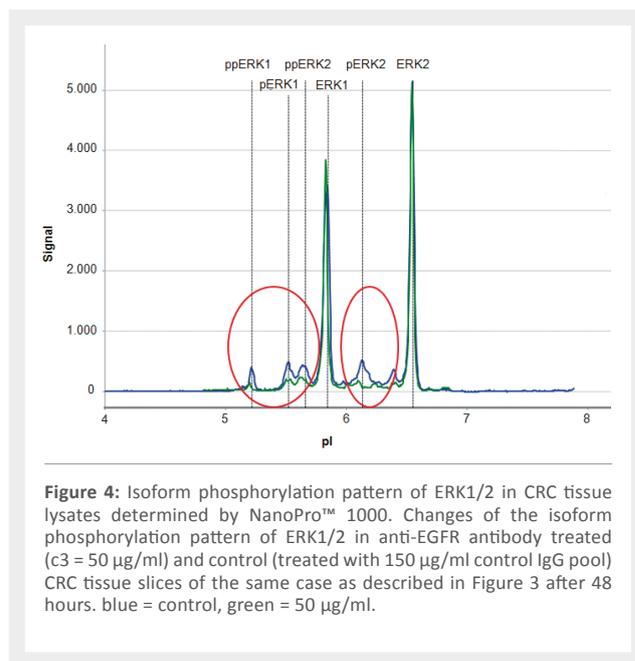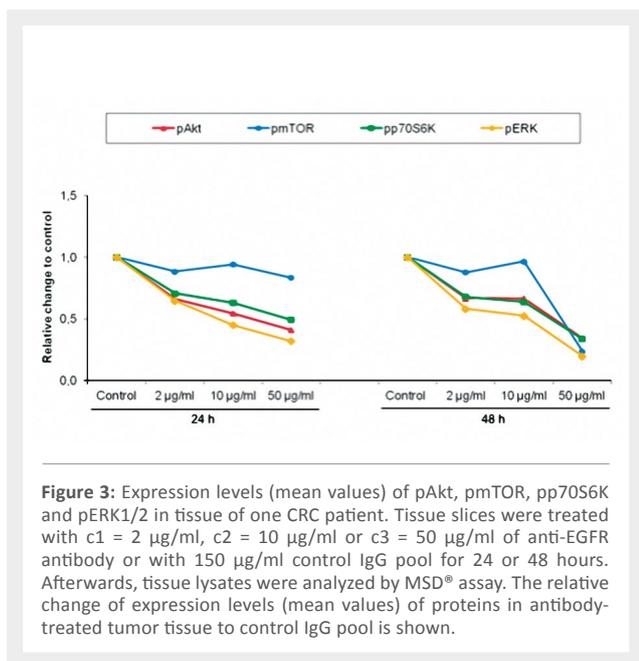

Figure 2: Representative immunohistochemical (IHC) staining results of control-IgG pool (A, C, E) and anti-EGFR antibody (c3 = 50 µg/ml; B, D, F) treated tissue from a CRC patient after 48 hours. IHC staining was performed against EGFR (A, B), Ki67 (C, D) and pERK (pMAPK; E, F).

As readout of treatment effects, the expression levels of the target proteins, pAkt, pmTOR, pp70S6K and pERK1/2 were measured in antibody-treated tumor samples using the MSD® technology. Evaluation of the protein activity levels showed that their expression pattern differed between the individual cases, thus illustrating the high heterogeneity of tumors and differences between individual patients. For two cases inhibitory drug effects could be observed after antibody treatment, indicated by decreased expression levels of the selected key proteins. One case in particular showed a dose-dependent reduction of pathway activity (Figure 3).

IHC studies on pERK1/2 were performed to validate MSD® data. Expression patterns demonstrated that both signaling proteins decreased after anti-EGFR antibody treatment, confirming the MSD® results and underlining the functionality and inhibitory effects of the antibody in precision cut cancer tissue slices (Figure 2).

Figure 3: Expression levels (mean values) of pAkt, pmTOR, pp70S6K and pERK1/2 in tissue of one CRC patient. Tissue slices were treated with c1 = 2 μg/ml, c2 = 10 μg/ml or c3 = 50 μg/ml of anti-EGFR antibody or with 150 μg/ml control IgG pool for 24 or 48 hours. Afterwards, tissue lysates were analyzed by MSD® assay. The relative change of expression levels (mean values) of proteins in antibody-treated tumor tissue to control IgG pool is shown.

Figure 4: Isoform phosphorylation pattern of ERK1/2 in CRC tissue lysates determined by NanoPro™ 1000. Changes of the isoform phosphorylation pattern of ERK1/2 in anti-EGFR antibody treated (c3 = 50 μg/ml) and control (treated with 150 μg/ml control IgG pool) CRC tissue slices of the same case as described in Figure 3 after 48 hours. blue = control, green = 50 μg/ml.

To obtain a complex pattern of phospho isoforms, an analysis on ERK1/2 has been performed using the NanoPro™ 1000 technology. This new method allows the detailed identification of distinct isoform phosphorylations by separating the proteins according to their isoelectric point (pI). By using this method, we observed that the regulation of isoform phosphorylation patterns of ERK1/2 differed after treatment among patients, confirming the heterogeneity of individual responses among patients. As before, a dose-dependent regulation of total and phosphorylated ERK1/2 was observable in the precision cut cancer tissue slices after anti-EGFR antibody treatment, shown by reduced isoform phosphorylation levels (Figure 4).

Conclusion

In summary, the data demonstrated that functional drug effects of a monoclonal anti-EGFR antibody could be measured in precision cut cancer tissue slices. Therefore, the preclinical model based on cultured cancer tissue slices developed by Indivumed is suitable for examining the effects not only of classical chemotherapeutics and small molecules such as kinase inhibitors as we have shown recently, but also of larger molecules, such as therapeutic antibodies. Our results suggest that the model based on precision cut cancer tissue slices is suitable for a more comprehensive analysis of drug responses and in particular cellular responses to targeted drugs in a natural tumor microenvironment. Furthermore, the NanoPro™ 1000 technology can potentially be useful for identifying individual phosphorylation patterns of tumor cells, which may facilitate the discovery of predictive biomarkers to further improve personalized anti-cancer therapy.

Literature

1. Unger et al., Precision cut cancer tissue slices in anti-cancer drug testing. J Mol Pathophysiol. 2015; 4(3):108-121.
2. Unger et al., Drug profiling in precision cut cancer tumor slices: analysis of therapeutic antibodies in colorectal cancer. Int J Cancer Immunol Immun. 2015; 1: 101.

Additional Information

Please visit our website or contact us at the addresses below to learn more about Indivumed's drug profiling platform. Indivumed provides high quality drug profiling services on a fee-for-service basis.

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