Evaluation of EGFR induced on-target and target-mediated adverse effects in a microfluidic 3D human lung tumour – full thickness skin co-culture model

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Abstract

Microphysiological systems are increasingly contributing to the preclinical prediction of mode of action and adverse outcome pathways of new chemical and biological entities. The recent advent of robust human multi-organ-chip systems enables the establishment of co cultures of human drug target tissues with healthy organ equivalents prone to off-target effects of the respective drug.

Here we established a chip-based 5-day co-culture composed of human mucoepidermoid carcinoma H292 cell-based lung tumour spheroids and human skin equivalents. We investigated the impact of repeated Cetuximab exposure on the systemic behaviour of the co-culture and on individual tissue responses. We compared on-target antibody effects with response data for Afatinib – a small molecule benchmark drug. Finally, we investigated target-mediated adverse effects on healthy skin equivalents.

Background

The epidermal growth factor receptor (EGFR) pathway plays a vital role in the pathogenesis and progression of non small cell lung cancer (NSCLC).

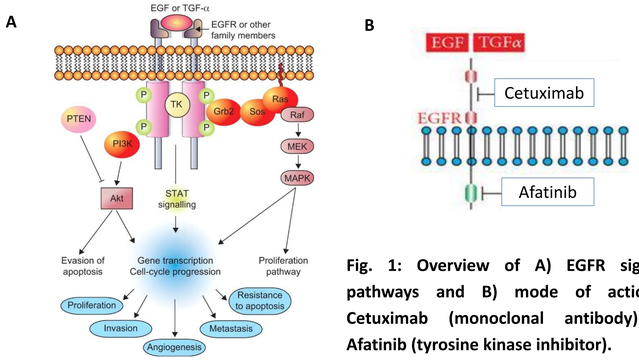
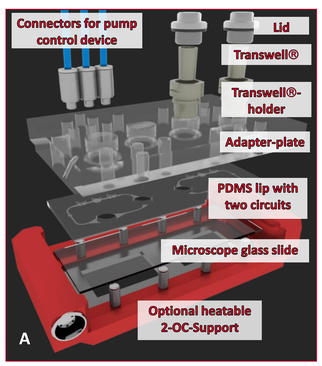


Fig. 1: Overview of A) EGFR signaling pathways and B) mode of action of Cetuximab (monoclonal antibody) and Afatinib (tyrosine kinase inhibitor).

Experimental procedure

Using the 2-Organ-Chip (2-OC) 15 lung tumor spheroids (each 10.000 H292 cells) and one 24-well full-thickness skin model per circuit have been co-cultured for 5 days. After 24 h compounds were applied into the microfluidic for 4 days consecutively. Responsive dose was detected by treatment of H292 cells in monolayer for 96 h followed by a MTT assay.



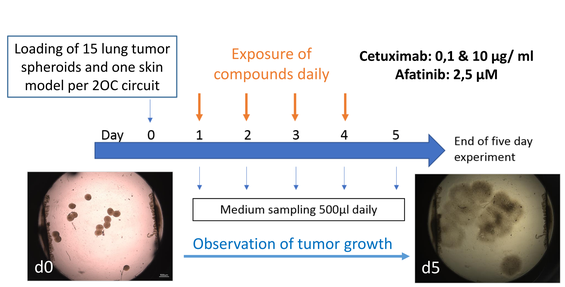


Fig. 2: Overview of experimental procedure. A) Schematically view of 2-Organ Chip B) Timeline of experiment including loading day, exposure days, sample volume and tumor expansion.

Cetuximab induced effects on co-cultured tissues

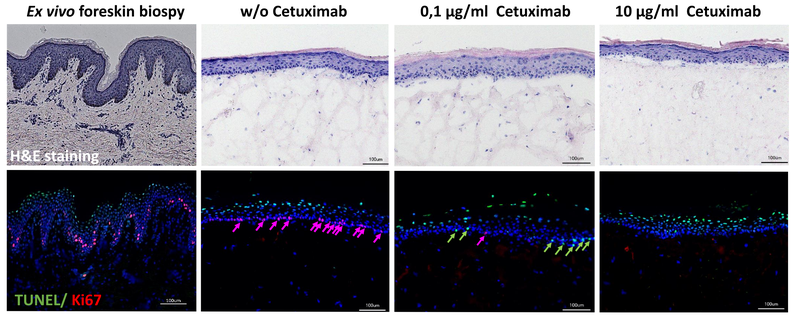


Fig. 3: Adverse effects on skin models shown by histological analysis. Upper row: H&E staining of cryosections, lower row: TUNEL+ Ki67 staining. Keratinocyte deorganization within the epidermis and loss of Ki67⁺ cells within the stratum basale indicating strong effect of monoclonal antibody treatment on skin models.

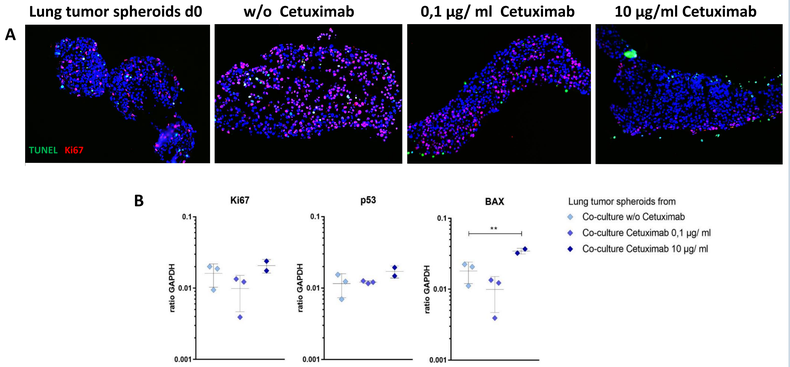


Fig. 6: On-target effects on lung tumor spheroids. A) TUNEL (green, apoptotic cells) + Ki67 (red, proliferative cells) IHC staining B) gene expression analysis after 5-days of chip co-cultivation. A statistically significant induction of pro-apoptotic marker BAX could be detected. *** P<0,005.*

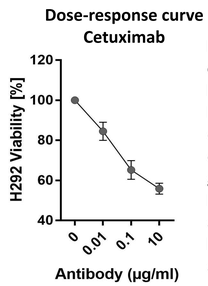


Fig. 4: Dose response curve for Cetuximab for H292 cells in monolayer. By applying three different antibody concentrations a similar growth inhibition for H292 cells was detected as shown already in literature (Desnoyers et al. 2013).

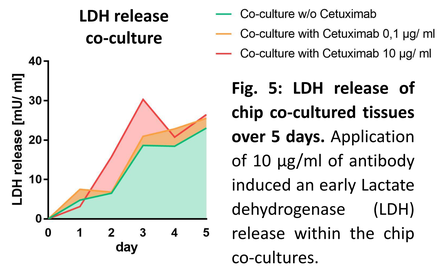


Fig. 5: LDH release of chip co-cultured tissues over 5 days. Application of 10 µg/ml of antibody induced an early Lactate dehydrogenase (LDH) release within the chip co-cultures.

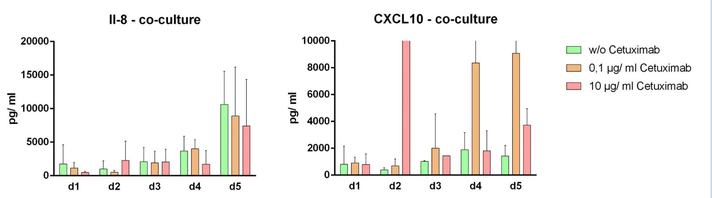


Fig. 7: Chemokine release of chip co-cultures. The release of chemokines (Interleukin8 [Il-8] and CXCL10) was measured in medium samples taken daily. Il-8 was reduced in the Cetuximab treatment. Conversely, CXCL10 production was increased compared to non-treated circuits.

Afatinib induced effects on co-cultured tissues

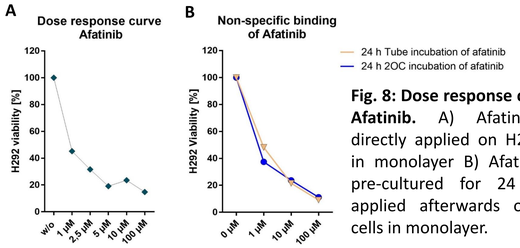


Fig. 8: Dose response curve for Afatinib. A) Afatinib was directly applied on H292 cells in monolayer B) Afatinib was pre-cultured for 24 h and applied afterwards on H292 cells in monolayer.

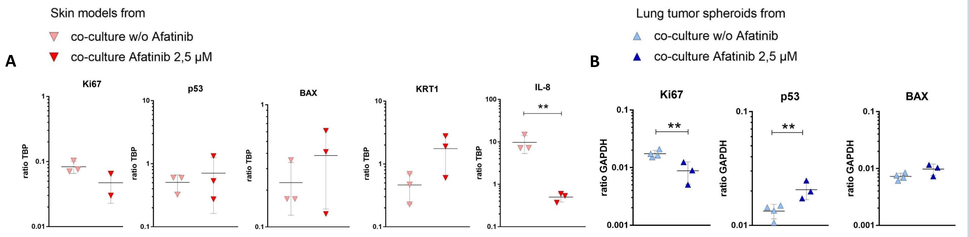


Fig. 9: Gene expression analysis of A) skin models B) lung tumor spheroids after 5-days of chip co-cultivation. Treatment of Afatinib induced combined effects on both tissues on the gene expression level using the 2-OC. *** P<0,005.*

Conclusion

Here, we repeatedly administered the EGFR targeting compounds to a novel microfluidic human 3D tumor – skin co-culture model to generate on- and target-mediated adverse data of tumor tissue and skin simultaneously. Four day repeated dose systemic anti – EGFR antibody administration supported detection of basal layer keratinocyte damage in the full thickness skin model at immunohistochemistry level and tumor response in the 3D tumor compartment on gene expression level of the microfluidic system. Treatment with the tyrosine kinase inhibitor induced similar effects on both tissues. Overall, the results indicate the potential of the Multi-Organ-Chip technology to provide a platform for evaluation of the therapeutic window of drug candidates.