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

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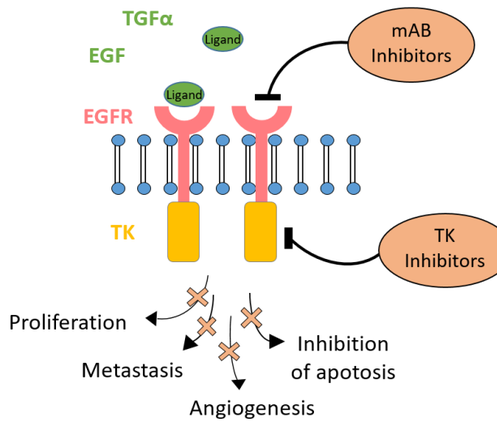
Introduction

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. We established a chip-based 5-day co-culture composed of human mucoepidermoid carcinoma H292 cell-based lung tumour spheroids and human skin equivalents. As the epidermal growth factor receptor (EGFR) pathway plays a vital role in the pathogenesis and progression of non small cell lung cancer (NSCLC) 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.

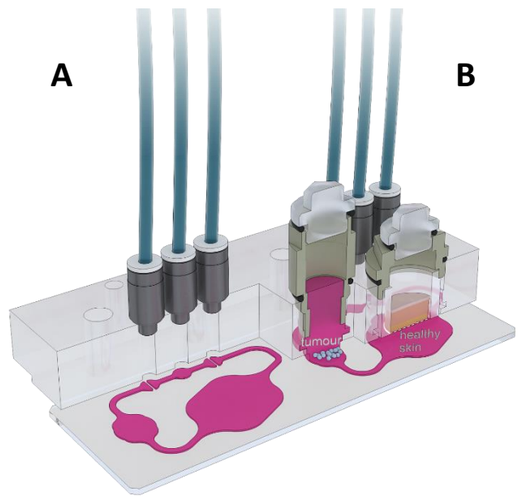


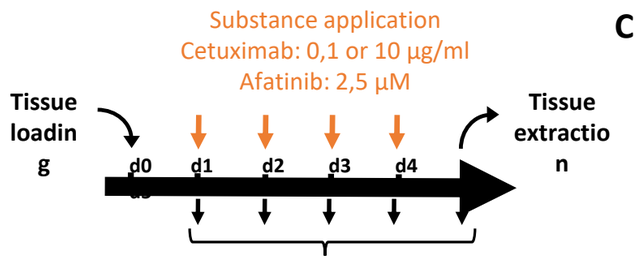
Inhibition strategies:

- Anti-EGFR monoclonal antibody (mAB) inhibitors (e.g. Cetuximab)
- EGFR-Tyrosine kinase (TK) inhibitors (e.g. Afatinib)

Experimental procedure

15 lung tumor spheroids (each 10.000 H292 cells) and one 24-well full-thickness skin equivalent have been co-cultured using the 2-Organ-Chip for 5 days. Cetuximab or afatinib have been repeatedly applied into the culture medium. Prior to the chip culture H292 cell line sensitivity was detected using a standard proliferation assay to define a dose-response curve.





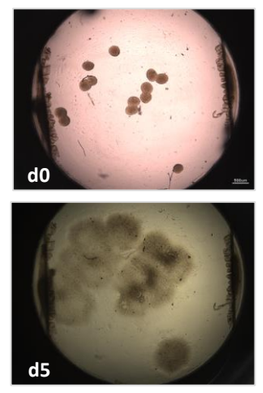


Figure 1 describes the experimental procedure for the chip culture at a glance. A) Cross-section of the 2-Organ Chip illustrating tumour and skin compartments. B) Timeline of chip experiment. C) Arrangement of tumour spheroids on day 0 and 3D fried egg shape after five days of chip co-culture.

Results

Cetuximab effects on co-cultured tissues

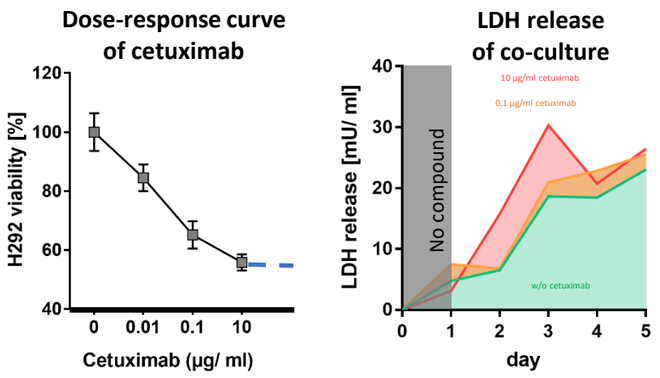


Fig. 2: Dose response curve of cetuximab. Static monolayer proliferation assay showed a dose dependent decrease of cell viability of up to 56% at the highest dose of 10 µg/ml. A higher dosage wouldn't increase the amount of cell death (see Desnoyers et al. 2013).

Fig. 3: LDH release of chip co-cultured tissues over 5 days. Application of 10 µg/ml of antibody induced a direct Lactate dehydrogenase (LDH) release indicating cell death.

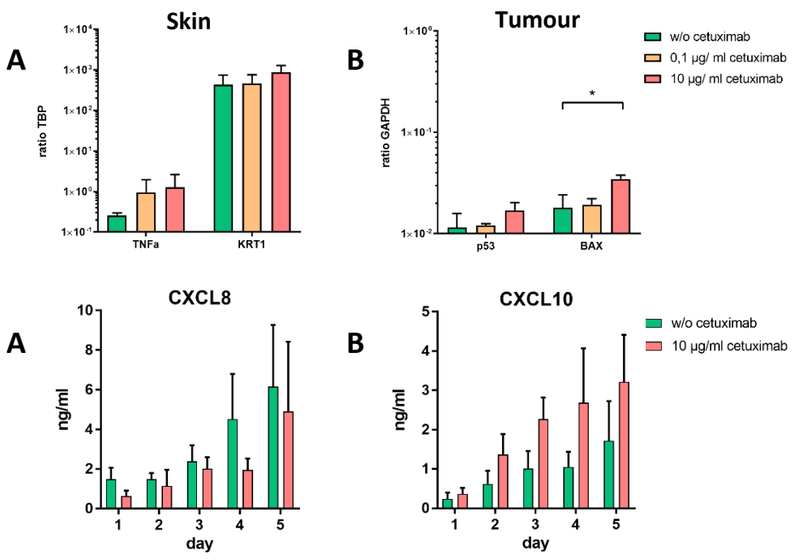


Fig.4 Gene expression analysis. A) TNFα and Keratin 1 gene expression of skin. B) tumour protein p53 and apoptosis regulator BAX gene expression of tumour. Statistically significant induction of pro-apoptotic marker BAX could be detected within cetuximab treated group .** P<0,05.*

Fig. 5: Chemokine release of chip co-cultures. The release of chemokines (Interleukin8 [Il-8] and CXCL10) was measured in medium samples taken daily. Notable reduction of Il-8 and increased CXCL10 production in treated compared to non-treated group could be detected as reported in literature.

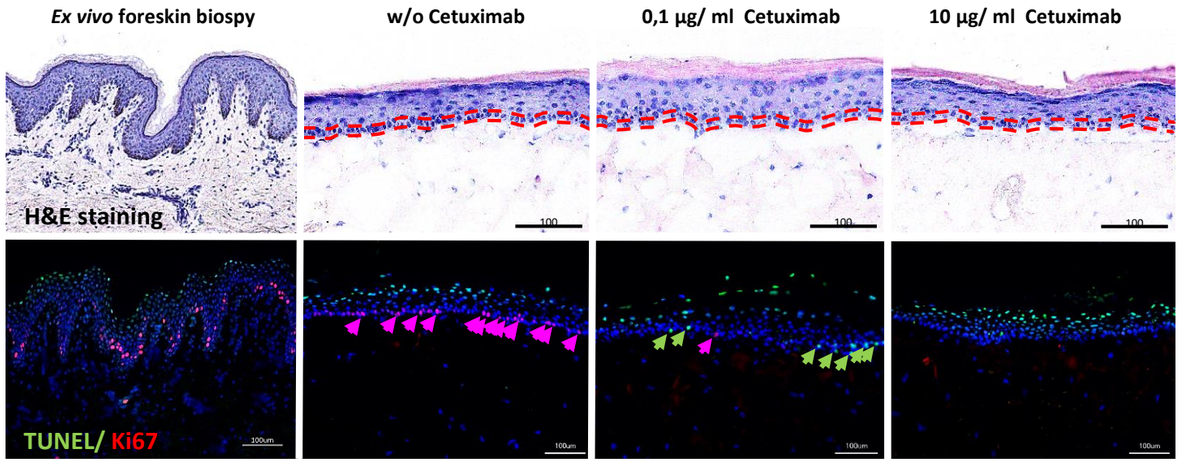


Fig. 6: Adverse effects of cetuximab on skin equivalents shown by histological analysis. H&E staining (upper row) revealed an irregular arrangement of keratinocytes in the stratum basale of treated skin equivalents (framed within dotted lines) combined with a complete loss of Ki67⁺ proliferative cells and a partially increased amount of apoptotic TUNEL⁺ cells (green; indicated by colored arrows; lower row).

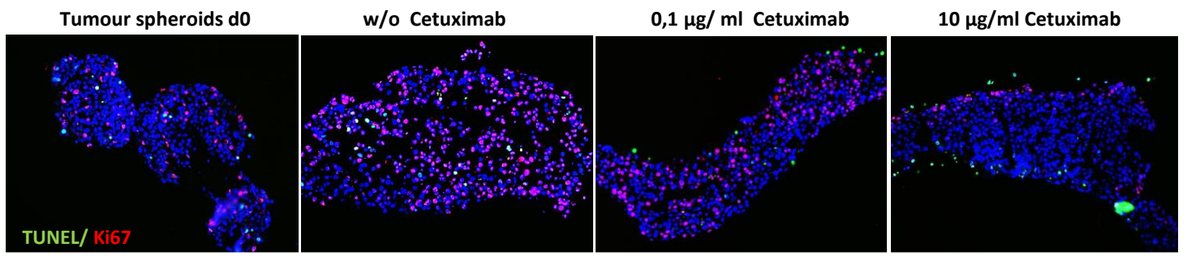


Fig. 7: On-target effects on lung tumor spheroids shown by histological analysis. Cetuximab reduced amount of Ki67⁺ proliferative cells (red) inside the tumour spheroids when comparing treated with untreated tumour tissues. A TUNEL staining for apoptotic cells (green) did not indicate an increase of cell death during chip culture.

Afatinib effects on co-cultured tissues

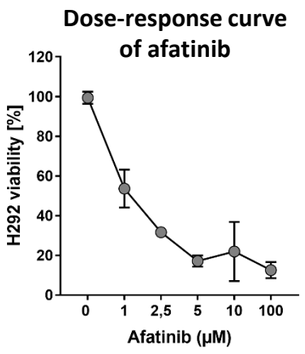


Fig. 8: Dose response curve of afatinib. Static monolayer proliferation assay showed a dose dependent decrease of cell viability of up to 15% at the highest dose of 100 µM. For treatment of chip co-cultures a concentration of 2,5 µM was used.
No decrease of afatinib activity was detected when pre-cultured in the 2-Organ-Chip prior to the monolayer assay indicating no diffusion of afatinib into the PDMS layer of the 2-Organ-Chip.

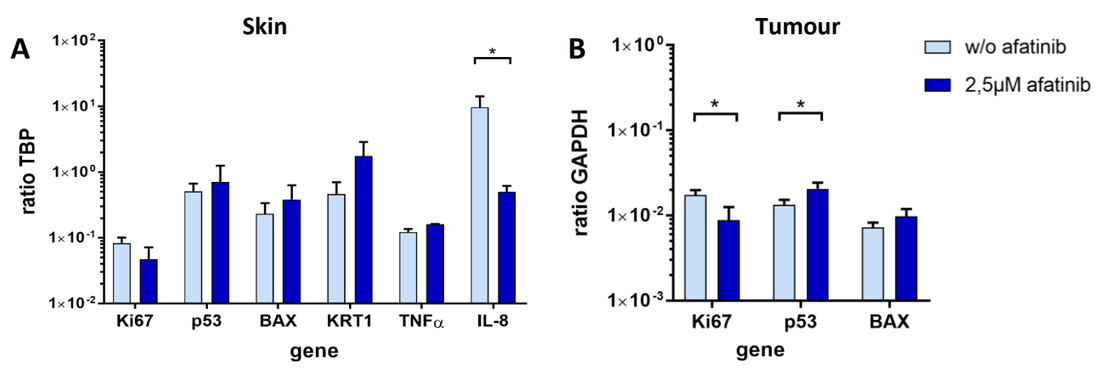


Fig. 9: Gene expression analysis. A) skin, B) lung tumor gene expression. Treatment of afatinib induced considerable differences in the gene profiles of the treated group in comparison to the untreated group. ** P<0,05 using two-tailed unpaired t-test. Data shown as mean +SD.*

Conclusion

Here, we repeatedly administered EGFR targeting compounds to a novel microfluidic human 3D tumor – skin co-culture model to generate on- and target-mediated adverse effects of tumor tissue and skin simultaneously. Four day repeated dose anti – EGFR antibody administration supported detection of basal layer keratinocyte damage in the full thickness skin model and pro-apoptotic tumor effects.

Treatment with the tyrosine kinase inhibitor afatinib induced similar effects on both tissues on the gene expression level. 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.

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