Cancer-associated fibroblasts promote drug resistance in *ALK*-rearranged lung adenocarcinoma cells through upregulation of cholesterol biosynthesis.

A. Daum[1,3], M.A. Schneider[3,4], M. Meister[3,4], M. Thomas[3,4,5] and H. Sültmann[1,2,3]

[1]Div. of Cancer Genome Research, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg; [2]German Cancer Consortium (DKTK); [3]German Center for Lung Research (DZL), TLRC Heidelberg; [4]Translational Research Unit, Thoraxklinik at University Hospital Heidelberg, Heidelberg, [5]Department of Thoracic Oncology, Thoraxklinik at University Hospital Heidelberg, Heidelberg

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Research for a Life without Cancer

Introduction

Targeted therapy interventions provide compelling response rates in *ALK*-rearranged lung adenocarcinoma patients. The occurrence of resistances, however, still poses a major clinical challenge. Several lines of evidence support a role of the tumor microenvironment (TME), especially of cancer-associated fibroblasts (CAFs), to such treatment insensitivities, while underlying mechanisms remain poorly understood. This project therefore aims to uncover the molecular networks shaping the susceptibility of lung cancer cells towards ALK inhibition in 3D co-culture settings.

Modeling of the TME-complexity with 3D co-cultures

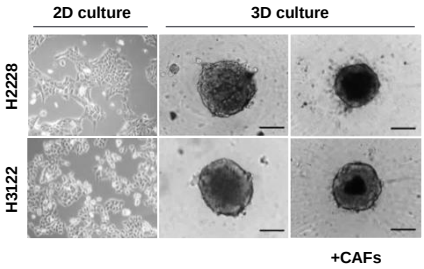


Figure 1. 3D spheroid co-culture models of H2228 (*EML4-ALK* v3) and H3122 (*EML4-ALK* v1) NSCLC cells in combination with CAFs, respectively. Scale bar: 200 µm.

Results

Reduced apoptosis of TKI-treated NSCLC spheroids upon co-cultivation with CAFs

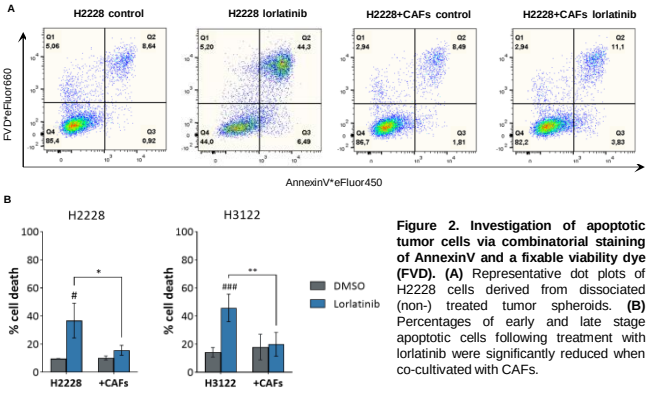


Figure 2. Investigation of apoptotic tumor cells via combinatorial staining of AnnexinV and a fixable viability dye (FVD). (A) Representative dot plots of H2228 cells derived from dissociated (non-) treated tumor spheroids. **(B)** Percentages of early and late stage apoptotic cells following treatment with lorlatinib were significantly reduced when co-cultivated with CAFs.

CAFs modulate cell cycle progression of TKI-treated NSCLC spheroids

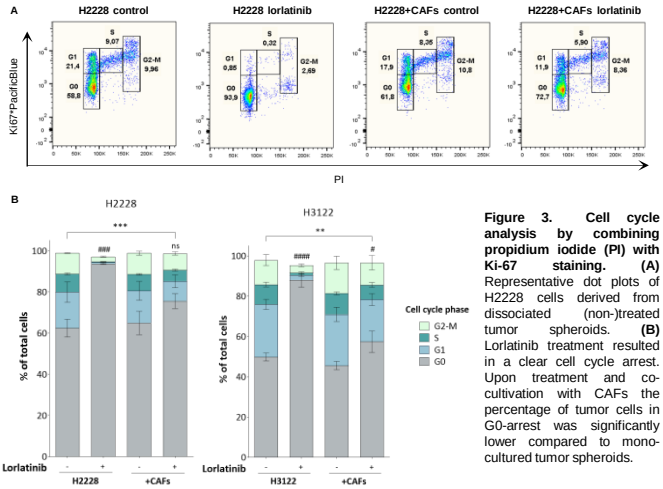


Figure 3. Cell cycle analysis by combining propidium iodide (PI) with Ki-67 staining. (A) Representative dot plots of H2228 cells derived from dissociated (non-)treated tumor spheroids. **(B)** Lorlatinib treatment resulted in a clear cell cycle arrest. Upon treatment and co-cultivation with CAFs the percentage of tumor cells in G0-arrest was significantly lower compared to mono-cultured tumor spheroids.

Results

scRNA-Seq reveals distinct clusters for (non-)treated NSCLC cells and CAFs

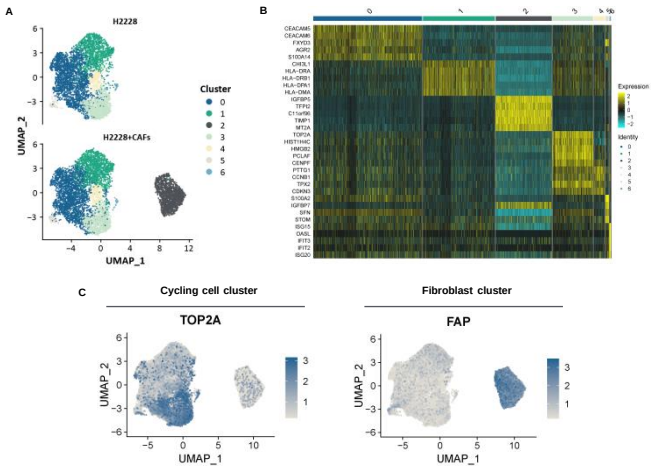


Figure 4. ScRNA-Seq of (non-)treated mono- and co-cultured NSCLC spheroids. (A) UMAP dimensionality reduction analysis identified seven major clusters. Each dot represents a single cell. **(B)** Heatmap of the top 5 most differentially expressed genes in each cluster from Fig. 4A. **(C)** Feature plots depicting gene expression of genes specific for cycling cells and fibroblasts, respectively.

Interference with TKI-induced dysregulation of tumor cell cholesterol biosynthesis by CAFs

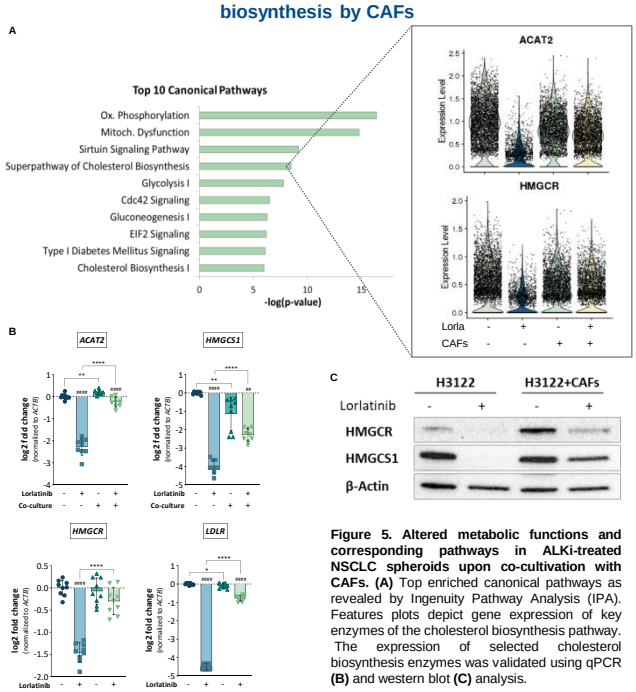
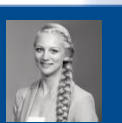


Figure 5. Altered metabolic functions and corresponding pathways in ALKi-treated NSCLC spheroids upon co-cultivation with CAFs. (A) Top enriched canonical pathways as revealed by Ingenuity Pathway Analysis (IPA). Features plots depict gene expression of key enzymes of the cholesterol biosynthesis pathway. The expression of selected cholesterol biosynthesis enzymes was validated using qPCR **(B)** and western blot **(C)** analysis.

Conclusion and outlook

Our findings indicate that CAFs form an environment which protects H2228 and H3122 cells from treatment with ALKi. This was demonstrated by significantly less apoptosis and a clearly reduced G0-cell cycle arrest. ScRNA-Seq revealed an enrichment of metabolism-related functions and pathways in TKI-treated tumor spheroids in presence of CAFs. Additional investigations using metabolomic and phosphoproteomic technologies are ongoing. The functional impact of obtained alterations will be further validated *in vitro* and *in vivo* to decipher distinct targets and corresponding pathways, responsible for therapy resistances impelled by the crosstalk of NSCLC cells with CAFs.



Contact: a.daum@dkfz.de

dkfz. German Cancer Consortium Core center Heidelberg | DKTK Deutsches Konsortium für Translationale Krebsforschung | NCT | DZL TLRC