# Molecular Characterization of the Tumour-Stroma Crosstalk Using a Novel 3D Co-Culture *In Vitro* Model*

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## Introduction

Carcinomas are highly complex structures composed of genetically altered tumor cells and stromal cells. Normal fibroblasts (NFs), cancer-associated fibroblasts (CAFs), endothelial cells, pericytes and inflammatory cells are the major constituents of stroma. The resulting molecular heterogeneity influences the way tumor cells migrate, proliferate and survive during tumor progression. Even resistance to therapeutic intervention has been linked with the molecular crosstalk between tumor cells and stromal cells. In addition, there is preliminary experimental evidence that CAFs might also play a role in stemness and formation of the metastatic niche. Recently, it has been shown that normal dermal fibroblasts can be in fact "educated" by carcinoma cells to express pro-inflammatory genes which orchestrate tumor-promoting inflammation in an NF-κB-dependent manner (Hanahan *et al*. 2010). Therefore, targeting CAFs is an important novel therapeutic concept.

To date, however, very little is known about the molecular signals involved in these afferent and efferent crosstalk pathways. With the aim to identify the underlying molecular mechanisms ("epigenetic make-up of CAFs") in the crosstalk between non-small cell lung cancer (NSCLC) epithelial cells and their corresponding CAFs a novel and complex 3D organotypic (spheroid) *in vitro* cell culture model system was established. This model is thought to much better recapitulate the *in vivo* situation as it reflects the complexity and dynamics of human tumors more faithfully than 2D monotypic monolayer cultures.
Transcription profiling of pairs of primary NFs and CAFs derived from NSCLC patients identified CAF-specific signature genes that are mainly involved in MAPK-signaling and focal adhesion interactions. Our data support the potency of the established 3D *in vitro* cell culture model even though fetal or embryonic FBs, respectively, have been used so far for co-cultivation with different NSCLC cell lines.

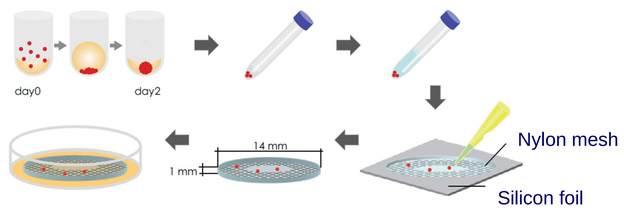
## Materials & Methods

### Spheroid formation and Collagen Gel Culture

- Tumor cells were seeded in a 96-well plate (U-shaped) and incubated with growth medium (+10% FCS) containing 12 mg/ml methylcellulose.
- Multicellular spheroids were harvested after 72h and mixed with a collagen I solution w/wo FBs and afterwards poured into a casting mould containing a nylon mesh. After 1h incubation at 37°C and 5% $CO_2$ the polymerized gel with its embedded nylon mesh was transferred into a 24-well plate and cultured with growth medium (+10% FCS) for up to three days (Fig.1, Dolznig *et al.* 2011).

### Spheroid Formation: Experimental Setup

Fig.1

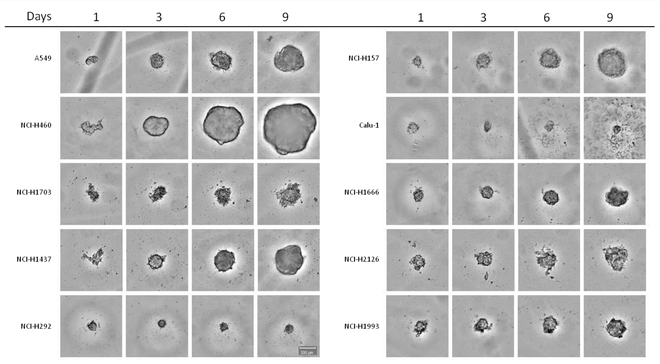



## Results

### Spheroid Formation and Cell-to-Matrix Interaction

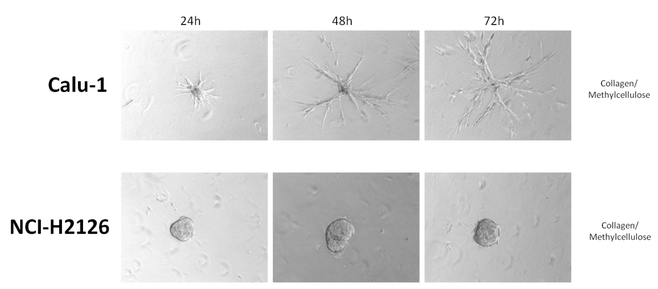
- To test the ability of multicellular 3D spheroid formation, different NSCLC cell lines were tested (Fig.2).
- Overall shape and growth behaviour of the spheres turned out to be cell line specific; i.e. NCI-H460 cells formed well-defined and fast growing spheroids, others revealed spotted characteristics or a "resting" phenotype, such as NCI-H292.

Fig.2




- After embedding the 3D spheroids into a collagen I matrix (see protocol Fig.1), invasion could be observed in some of the 3D spheroid models after several days of incubation, i.e. Calu-1 (Fig.3) ➔ strong cell-to-matrix interaction of Calu-1 cells and collagen I.

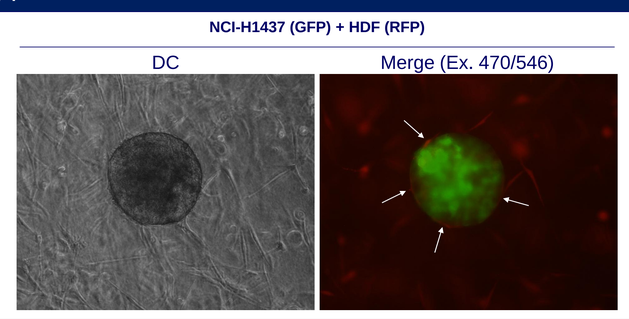
Fig.3




### Cell-to-Cell Interaction between Tumor Spheroids and FBs

- Co-cultivation of tumor spheroids together with FBs
- Utilization of different FB cell lines: HDFs (human dermal fibroblasts; fetal), WI-38 (human lung fibroblasts; embryonic) and IMR-90 (human lung fibroblasts; fetal)
- Strong cell-to-cell interactions in a co-culture of NCI-H1437 spheroids and HDFs (Fig.4)
- Tumor spheroids attract HDFs
- HDFs are physically connected to tumor spheroids

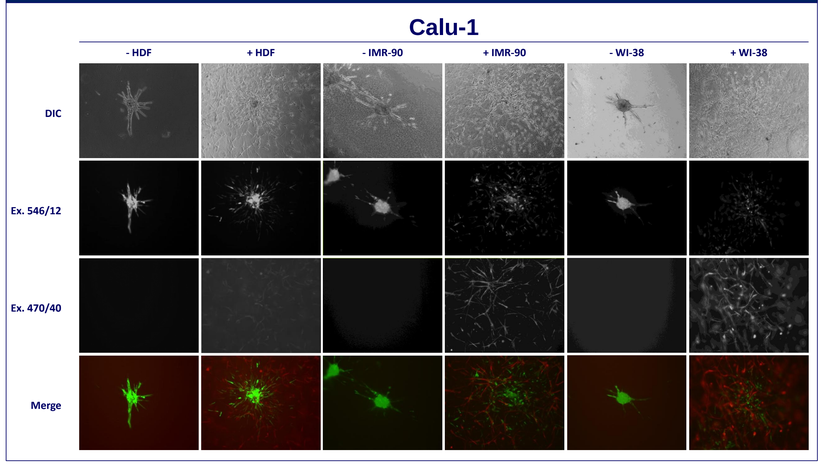
Fig.4




### Influence of FBs on Invasion

- After 24h of co-cultivation Calu-1 exhibited a strong dependency on HDFs for invasion compared to a Calu-1 mono-culture (Fig.5).
- Mono-cultures formed invasive asterisks-like spheroids loosing their spherical phenotype.
- Co-cultivation led to dissemination of single cells, leaving a highly scattered phenotype.
- Interestingly, when co-cultivating Calu-1 spheroids together with lung fibroblasts such as IMR-90 and WI-38, the invasion effect seemed much more prominent than observed with HDFs.

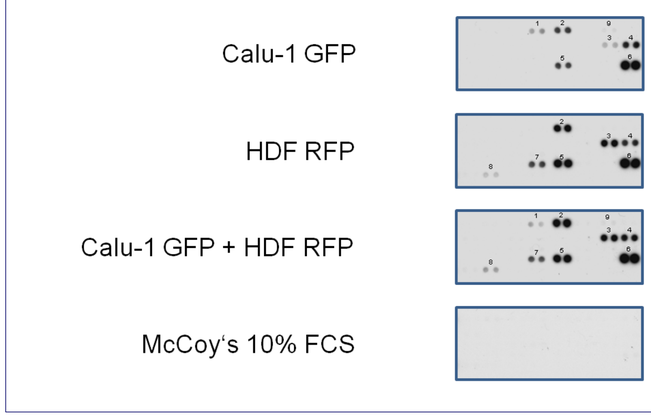
Fig.5




### Cytokine & Chemokine Cross-talk

- Supernatants from Calu-1, HDFs and Calu-1/HDFs co-culture were incubated with the cytokine protein array (R&D, Fig.5).
- Next step: Study influence of knock down of these genes (si or shRNA) on the migration behaviour of Calu-1 in the 3D co-culture system.

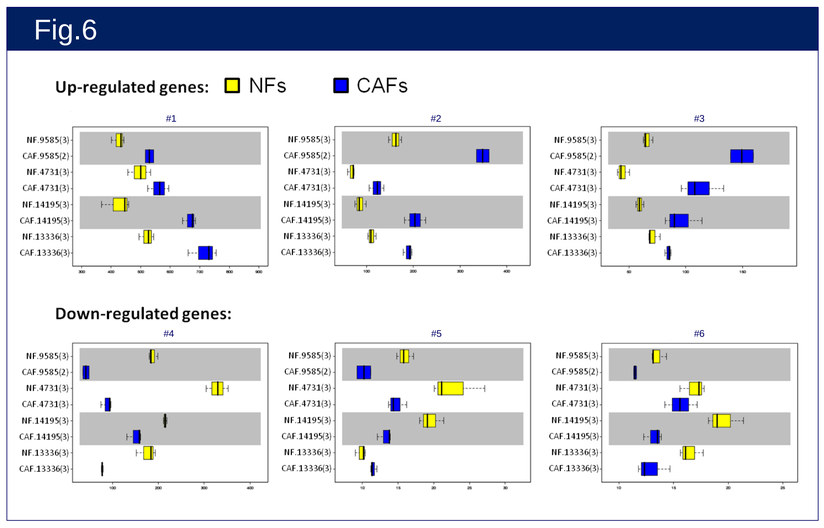
Fig.5




### Preliminary Data from CAF-Expression Profiling Study (Fig.6)

- CAFs derived from patient #14195 and #9585 exhibit overlap with published gene signature (Navab et al. 2011)
- Signature genes are mainly involved in MAPK-signaling, focal adhesion interactions
- Data from patient #4731 and #13336 are less clear (mixture of NFs and CAFs?)

Fig.6




## Next Steps

- Immortalization of CAFs by h-TERT and co-cultivation with NSCLC spheroids to further analyze the molecular crosstalk events
- Expression profiling of additional NFs/CAFs patient pairs for dataset enrichment
- Expression profiling of co-cultivated tumor cells and FBs/CAFs

## Literature

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