CO-SPHEROID ANALYSIS REVEALS ATTENUATING EFFECT OF HS27A STROMA CELLS ON DLD1 COLON CARCINOMA SUSCEPTIBILITY TO MEK KINASE INHIBITOR TRAMETINIB

Claudia Hoffmann, Daniel Feger, Oliver Siedentopf, Holger Weber, Sarah Umber and Jan E. Ehlert

Reaction Biology Europe GmbH, Freiburg, Germany

Introduction

In cancer treatment, stroma-derived microenvironmental cues are suspected to exert an adversary impact on anti-cancer drug efficacy. In order to take such influences into account in cellular drug screening campaigns, we have set up a high-throughput-compatible co-spheroid model, enabling the analysis of various combinations of cancer and stroma cell lines. This assay system allows for cell analysis in a three-dimensional (3D) close-to-physiological environment with simultaneous detection of cancer and stroma cell viability based on differential luciferase cell-labelling.

Methods

Spheroid-Assay (see scheme in figure 1):

Tumor cells or/and stroma cells were seeded in growth medium containing methyl-cellulose in low attachment round bottom 96 well plates. After 24 h, when cells had formed spheroids, compounds were added to the cells. The spheroids of the day 0 control plate were lysed and frozen. After an incubation time of 72 h treated cells were lysed and frozen. Subsequently luciferase activity of all samples was analyzed.

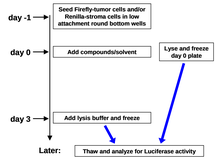

Figure 1: Scheme of the spheroid assay procedure.

Measurement of luciferase activity in co-spheroids:

Lysed and frozen cells were thawed and completely lysed by mechanical sheering, which was done by extensive up and down pipetting. 2x 40 µl of the lysates were transferred on two white flat bottom 96well plates and incubated with equal volumes of the corresponding luciferase substrate (each for Renilla and Firefly luciferase). After 30 min the luminescence is measured using a plate reader.

Tumor and stroma cells forming mono-spheroids

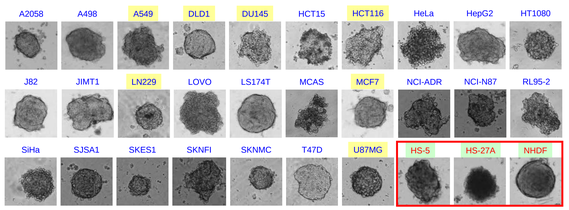

Figure 2:

Spheroids of **tumor** or **stroma** cells. Spheroids of 1000 cells were photographed 24 h after seeding. Tumor cells were stably transduced with Firefly luciferase and stroma cells with Renilla luciferase.

Effect of Trametinib on mono-spheroids

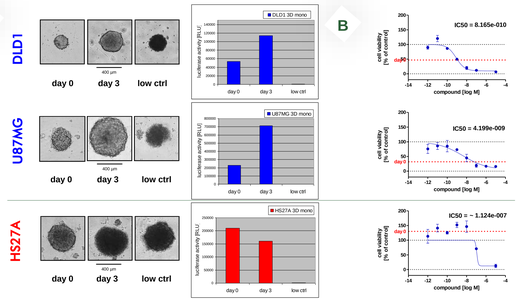

Figure 3:

Treatment of tumor and stroma cell spheroids with Trametinib. **(A)** Spheroids of DLD1 tumor cells, U87MG tumor cells and HS27A stroma cells were photographed at day 0, day 3 (high control) and after treatment with Staurosporine for 72 h (low control). Viability of the spheroids was analyzed by luminescence measurement. **(B)** Spheroids were treated for 72 h with indicated concentrations of Trametinib. Viability is presented in percentage of high control (untreated spheroids).

Differential cell type detection in co-spheroids

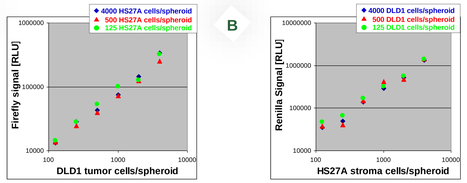

Figure 4:

Correlation between luciferase activity and cell number in co-spheroids. Firefly luciferase transduced DLD1 tumor cells and Renilla luciferase transduced HS27A stroma cells were co-cultured as spheroids with different cell ratios. Activity of **(A)** Firefly luciferase in DLD1 tumor cells and of **(B)** Renilla luciferase in HS27A stroma cells was analyzed by luminescence measurement.

Growth in co-spheroids depends on cell type ratio

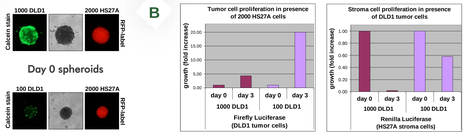

Figure 5:

Interaction between tumor and stroma cells in co-spheroids. **(A)** Photographs of co-spheroids with different content of DLD1 cells at day 0. **(B)** Growth of DLD1 and HS27A cells in co-spheroids of 1000 or 100 DLD1 cells with 2000 HS27A cells was analyzed after 72 h.

Effect of Trametinib on co-spheroids

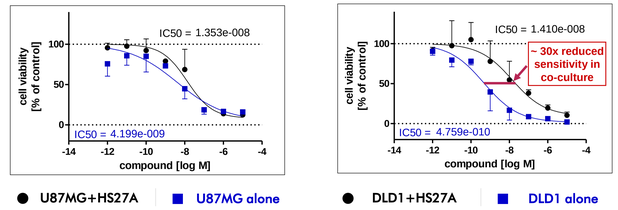

Figure 6:

Reduced sensitivity of DLD1 cells against Trametinib in co-spheroids . U87MG and DLD1 tumor cells were cultured in spheroids alone or together with HS27A stroma cells. Spheroids were treated for 72 h with indicated concentrations of the MEK kinase inhibitor Trametinib. Subsequently, viability of tumor cells was analyzed by measurement of Firefly luminescence.

Established co-spheroid models:

Firefly Luciferase labeled tumor cells	Renilla Luciferase labeled stroma cells: none	HS5	HS27	NHDF
none		X	X	X
A549	X	X	X	X
DLD1	X	X	X	X
HCT116	X	X	X	X
LN229	X	X	X	X
MCF7	O	O	X	X
U87MG	X	X	X	X

X : Good aggregation

O : Loose aggregation

Table 1:
Established co-spheroid models.

Conclusion

- Differential luciferase cell-labeling allows simultaneous detection of cancer and stroma cell viability in 3D co-spheroids.
- Viability of stroma cells in co-spheroids can strongly be influenced by the ratio of tumor and stroma cells.
- The sensitivity of DLD1 tumor cells against the MEK kinase inhibitor Trametinib is attenuated in the presence of HS27A stroma cells.
- Integrating co-spheroid models into early drug development allows to focus on compounds that remain active under microenvironmental conditions.