P-22

Augmented Drug Combination Efficacy in 3D and 3D Co-Culture Models of Colorectal Carcinoma

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Introduction: Targeted drug combinations have the potential to improve treatment activity through synergistic interactions, simultaneously allowing reductions in drug dosing, and by reducing drug resistance through cell viability inhibition on multiple levels. However, few targeted drug combinations have been approved and clinical drug attrition rates are still high. Current clinical trials often lack scientific evidence on the fitness of the drugs in the combination. This might be due to the fact that preclinical testing is often performed in simple two-dimensional (2D) cell cultures. Currently, three-dimensional (3D) cancer cell models are gaining attention as pre-clinical alternatives.
Aims: We compared drug combination efficacy and drug interactions between culture systems with various complexity, i.e. 2D, 3D and 3D co-cultures, to bridge the gap between *in vitro* research and possible translation to *in vivo* models.
Methods: We established the 3D and 3D co-culture (3D-CC) systems for a panel of human colorectal carcinoma (CRC) cell lines of various origin and genetic background to mimic the diversity observed in CRC patients. The 3D cultures formed compact heterogeneous shapes that increased in size over time and presented a remodeled periphery. The 3D-CC were established with clinically relevant number of endothelial cells (EC) and fibroblasts (FB), two cell types interacting with CRC cells in the tumor microenvironment. The 3D-CC were characterized with more heterogeneous shapes, and intra-spheroid organization of the FB and EC was cell-type dependent. The 3D-CC developed a core of fibroblasts with pockets of endothelial cells in close proximity or had FB and EC distributed throughout the spheroid.
Results: Three CRC cell types (HCT116, DLD1 and SW620) were selected for treatment optimization with CRC clinically relevant drugs, including the chemotherapeutic 5-fluorouracil and small molecule-based drugs, i.e. regorafenib and erlotinib, targeting VEGFR2-3/Ret/Kit/PDGFR/Raf and EGFR, respectively. We used cell metabolic activity as a readout in cell proliferation assays and incubated the cell cultures incubated with the drugs for 72 h. Interestingly, when comparing dose-response curves between 2D and 3D cultures, we observed a cell-type dependent increase in drug sensitivity in 3D versus 2D cultures for erlotinib. In the next step, we investigated the differences in drug combination efficacy and drug interactions between the CRC cell types and the culture systems at two dose levels, i.e. clinically relevant maximum plasma concentrations (MPCs) and at low dose (LD), representing a dose inducing approx. 20% of activity, to more optimally study drug interactions. We identified cell-type and culture-system dependent differences between the drug combinations. A key observation was a reduction in treatment efficacy of the 3D-CC and/or 3D cultures versus 2D cultures for 2-drug combinations at LD. Consistently, the increase in drug concentration from LD to MPC correlated with a shift from synergy/additivity towards additivity/antagonism. Moreover, some drug combinations did not gain activity when treatment was performed with MPC compared to LD. Retreatment resulted in striking cell type-dependent improved treatment efficacy, highlighting the potential of personalized low dose drug combinations.
Conclusion: Taken together, in our *in vitro* 3D (co-)cultures we detected culture system-dependent differences in drug combination efficacy and interactions and optimizing low-dose combinations with a personalized approach can improve current treatment options.

Keywords: colorectal cancer, 3D cultures, 3D co-cultures, endothelial cells, fibroblasts, drug combinations, drug interactions, retreatment