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Establishment of a comparative 2D/3D in vitro platform to study drug resistance in pancreatic ductal adenocarcinoma cell lines in mono- and co-culture with immortalized pancreatic stellate cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) is marked by an abundant desmoplastic stroma that likely contributes to so-called cellular adhesion mediated drug resistance (CAM-DR). Pancreatic stellate cells (PSC) orchestrate a complex tumor promoting interplay in the stromal compartment with TGF β being an important mediator. Multicellular spheroids are three-dimensional in vitro models that bear a striking resemblance to intercapillary tumor microregions. The thesis delineates the development of a spheroid-based in vitro drug-testing platform taking into account the mutual crosstalk between PDAC and PSC. **Methods:** As a first step, a spheroid co-culture method was developed and gradually adapted to an eight-day drug toxicity assay. Cell lines were cultured alone and in combination. Comparative drug toxicity testing in monolayer (2D) and spheroid (3D) cultures ensued comparing various means of viability assessment. Immortalized cell lines were used (PDAC: Panc1 +/- TGF β ; PSC: Klon2.2 eGFP). **Results:** Spheroid generation was reproducible. Growth characteristics and spheroid morphology pointed towards a significant interaction between the cell types in 3D co-culture. Comparative drug toxicity testing revealed a clearly reduced drug response in spheroids and postconfluent monolayers suggesting some degree of CAM-DR. TGF β was shown to confer an additional resistance to cells in 2D culture but there was no clearly attributable effect on treatment sensitivity in spheroids. It was neither possible to demonstrate an unequivocal effect of the co-culture of cancer cells with stellate cells on drug sensitivity. **Conclusions:** The study provides evidence of the feasibility of a high throughput in vitro drug toxicity assay employing multicellular spheroids from pancreatic cancer cells and stellate cells. The observed interaction between the cell types is intriguing and warrants further mechanistic evaluation. However, there remain a number of caveat regarding the assay's standardization and validation. These include the use of established cell lines, incomplete functional characterization of the mixed spheroids and concerns regarding the suitability of the resazurin-based fluorometric viability assay for drug toxicity assessment.