Three-Dimensional Organoid Systems for Modeling Colon Carcinoma Metastasis and Anti-Cancer Drug Development

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Introduction

Cancer treatments have advanced in recent years, yet current models for drug discovery and metastasis exploration poorly mimic human cancer in vivo. Research generally relies on animal systems and two-dimensional (2-D) cultures, both of which suffer from inadequacies. Animal models are inherently inaccurate when translated to human physiology, and 2-D cultures fail to recapitulate the three-dimensional (3-D) nature of in vivo tissues. When dosages are scaled for clinical use, they are often ineffective in patients. Alternatively, 3-D systems of human-derived cells can promote cell-cell and cell-matrix interactions, resembling in vivo conditions. Unfortunately, 3-D models are largely underutilized for cancer research. Here we present multiple host tissue-tumor systems for in vitro modeling of colon carcinoma metastasis with implications in exploring cancer biology and the potential to streamline the drug development pipeline.

Materials and Methods

In the first system, liver-tumor spheroids were created in rotating wall vessel (RWV) bioreactors using hyaluronic acid (HA)-based microcarriers combined with HEPG2 liver-based cells and HCT116 colon carcinoma cell cocultures. The progression of red fluorescent protein (RFP)-labeled HCT116 cells over time was assessed by microscopy. Second, a panel of cell adhesion proteins and metastatic markers were queried via IHC using the 3-D systems as well as traditional 2-D cultures. Finally, the response of the 3-D tissues to chemotherapy was assessed by treatment with the drug 5-fluorouracil (5FU) followed by MTS mitochondrial metabolic assays and IHC staining for activated caspase 3, a product of cellular apoptosis. The second system is comprised of a 2-compartment fluidics device, one containing an intestinal organoid incorporated with HCT116 cells and the other a liver organoid. This design allows in vitro modeling of metastasis from one organoid to another, which are integral in colon carcinoma progression. Within these 2 systems we characterized the motile and metastatic nature of the tumor foci, and the incidence of epithelial-to-mesenchymal transition (EMT) in several ways.

Results

In both spheroid and organoid systems, HCT116 cells proliferated rapidly as evidenced by RFP fluorescent tracking (Fig. 1). Additionally, the 2-chamber organoid fluidics system showed that 1) HCT116 cells can shed from the first organoid and enter circulation and 2) engraft in another organoid, invading via multicellular tendrils that break off and form spheroid-like aggregates that continue to migrate. IHC staining showed that in 2-D HCT116 cells appear epithelial, expressing membrane-bound ZO-1 tight junction markers and β-catenin, but not N-cadherin. Within 3-D liver constructs, HCT116 cells appear to undergo EMT, losing membrane-bound expression of ZO-1, β-catenin, E-cadherin, and vinculin, but expressing N-cadherin and matrix metalloproteinase 9. Following 5FU treatments, spheroids suffer a dose-dependent overall decrease in mitochondrial metabolism, despite maintaining their general morphology. Activated caspase 3 expression increased with 5FU concentration, mainly in HCT116 cells and not in the liver cells.

Fig. 1. Progression of metastatic HCT116 colon carcinoma within liver spheroids over time.

Discussion and Conclusions

These results demonstrate efficacy of these systems to model important characteristics of in vivo metastasis such as cancer cell proliferation, shedding and invasion, EMT and responsiveness to drugs. These systems represent potential models for exploration in metastatic cancer biology, more streamlined drug discovery, and testing of side effects of these drugs on the surrounding host tissues.

References List


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