Formation and Characterization of Colon Tumor Organoids from Primary Cells

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Statement of Purpose: Colorectal cancer is one of the most frequent and lethal types of cancer. Despite advances in recent decades, our knowledge of this disease is still limited. Novel and more physiological models are needed to better understand the disease and develop therapies. Organoids are three-dimensional (3D), self-organized multicellular structures generated by stem cells, adult stem cells, or cancer stem cells embedded in an extracellular matrix. Organoids closely mimic morphological and biological properties of their native tissues and offer a valuable tool for disease modeling. (1) The goal of this study is to establish organoids from primary human colon tumor cells and characterize them.

Methods: CNO375, conditionally reprogrammed colorectal cancer cells originally derived from a patient, were obtained through the NIH/NCI PDMR. Single cells were embedded in Matrigel on ice (growth factor reduced, phenol free) and seeded in 96-well plates (500 single cells per 25 µl of Matrigel per well). The Matrigel was polymerized for 10 minutes at 37°C.(2) Then, 200 µl culture medium F12 containing a rock inhibitor (Y-27632) was added. The culture medium was replaced every two days and the morphology and metabolic activity of organoids were evaluated daily. For the metabolic activity measurements, a Prestoblue reagent was added daily for 11 days to each well at 10% of total volume. For the characterization of the organoids, immunofluorescence was done using Ki67 for proliferation, cytokeratin 20 for differentiated cells as colonocytes, and beta catenin and actin as the markers for apical and basolateral side of epithelial cell monolayer defining a central lumen. Hoechst nuclear staining was also done. All the images were captured with confocal microscope and composite images were made in ImageJ.

Results: We found that the primary colorectal cancer cells formed organoids in Matrigel. Our morphological analysis showed an increase in the diameter of organoids from 87±1.47 µm on day 1 to 297±1.89 µm in diameter on day 11, i.e., a 3-fold increase in the diameter from day 1 to day 11 (Figure 1a). Metabolic activity of organoids progressively increased for a period of 11 days with a 13-fold increase in the fluorescence intensity from day 1 to day 11, indicating active proliferation in the organoids (Figure 1b). Immunofluorescence imaging of organoids on day 7 showed positive staining for cytokeratin 20, actin, and beta catenin (Figure 1c). At day 7, colonospheres were observed that are composed of epithelial cells with a central lumen-like structure, where the apical side oriented toward the lumen (actin labeling) and the basolateral side facing outwards (beta-catenin) were identified.

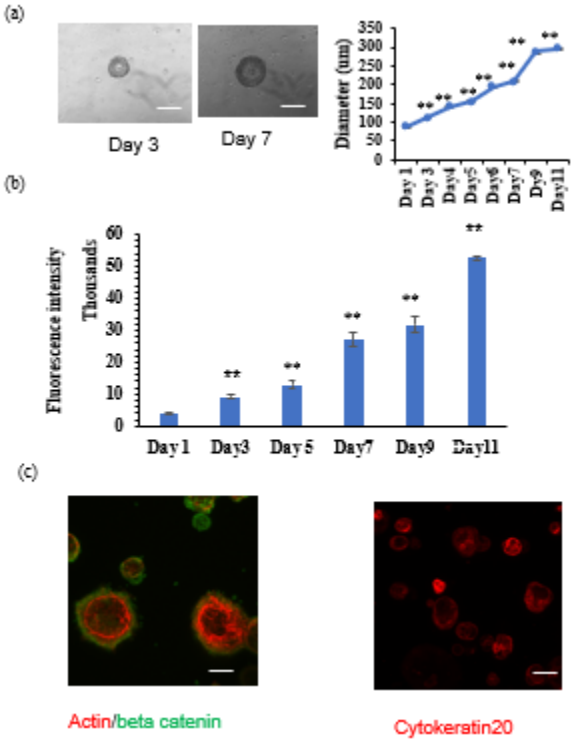

Figure 1: (a) Morphological images of organoids and quantified size of organoids during culture. (b) Quantified metabolic activity of organoids. (c) Immunofluorescence images of organoids labeled for actin, beta catenin, and cytokeratin 20. Scale bar is 30 µm. ** denotes p-value < 0.01.

Conclusion: We successfully formed colorectal cancer cell organoids from primary tumor cells in Matrigel. These organoids showed the characteristics of epithelial tumor markers as in colon tumors, offering a tool to study colon cancer biology and for drug discovery. We are currently evaluating the use of defined extracellular matrices as well as other components of the tumor microenvironment on the formation of colon cancer organoids and therapy responses to offer more complete and complex tumor models for research and drug discovery.

References:

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Acknowledgement: Financial support was provided by NIH grant CA216413.