Lawrence J. Ellison Institute for Transformative Medicine of USC

USC Center *for* Applied Molecular Medicine

A Window into Colorectal Cancer: Novel Tumor-on-Chip Model Combined with High-Content Imaging Offers Insight into Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is one of the deadliest cancers in the U.S., yet we still understand very little about the mechanisms behind this disease. We are developing a CRC Tumor-Chip model that can recapitulate the complex nature of tumorigenesis in order to increase our understanding of CRC and accelerate the discovery of effective new treatments.

The Organs-on-Chips technology maintains physiologically relevant aspects of organ structure and function by incorporating tissue compartments and mechanical forces to recreate *in vivo* mechanical forces (peristalsis) and fluid flow. The Intestine-Chip consists of two fluidic channels (endothelial cells and colon epithelial cells) separated by a porous membrane. We introduced fluorescently-labeled CRC cell lines onto the epithelium through optimization of seeding densities and culture conditions. CRC cells grew as 3-D clusters of varying sizes when seeded on the Chips compared to the 2-D elongated morphology traditionally observed in monolayer cultures.

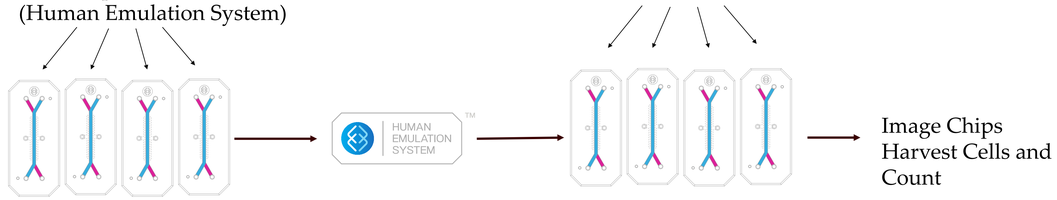
CRC Tumor-Chip model presents a challenge in assessing and quantifying cellular behaviors in response to perturbations to the system. To overcome this hurdle, we utilized a high-content imaging platform to quantify tumor cells within this heterocellular environment. Using this method, cancer cell growth was assessed and compared to traditional 2-D cell culture methods. Not surprisingly, CRC cells grew slower on the Chip than in conventional monolayer.

II. Methods for Determining CRC Cell Growth Rate on Chip

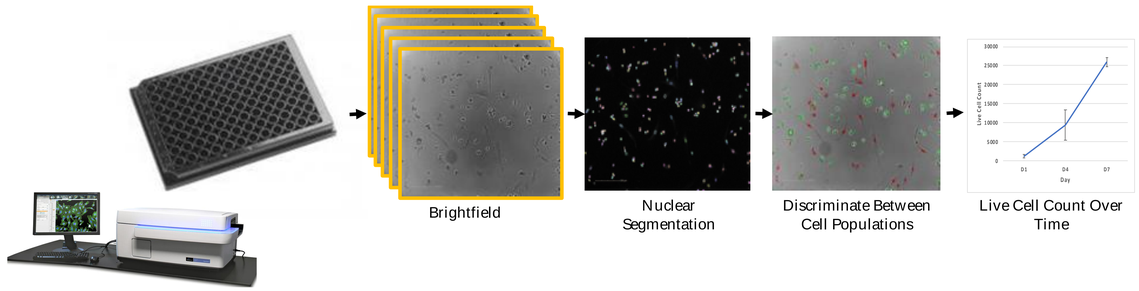
A. Schematic Overview of Experimental Workflow

1. ECM coat both channels
2. HUVEC to endothelial channel
3. Caco-2 to epithelial channel
4. Load chips into the instrument (Human Emulation System)

i. HCT116 GFP-H2B *or*
ii. HCT116 GFP-H2B + cancer associated fibroblasts (CAFs)



B. Image Analysis Workflow



Conclusions

We have modified the Intestine-Chip to develop a novel CRC-Tumor-Chip and established a method to quantify CRC phenotype (growth rate) in a heterogeneous cell population (HCT116, Caco-2, CAFs). Our preliminary findings suggest that CRC cell growth is slower on the Chip than in traditional cell culture conditions, which may be a better representation of tumor growth rates *in vivo*. Additionally, CRC cell growth on chips can be stimulated by the presence of patient derived CAFs. This work further emphasizes the need to study cancer progression in a more physiologically relevant model and has implications for patient treatment response.

Next Steps:

- Understand the role of mechanical forces on CRC cell growth
- Investigate the role of CAFs on CRC cell growth on the Chip
- Evaluate drug response in the Chip and compare to traditional cell culture methods

Acknowledgements

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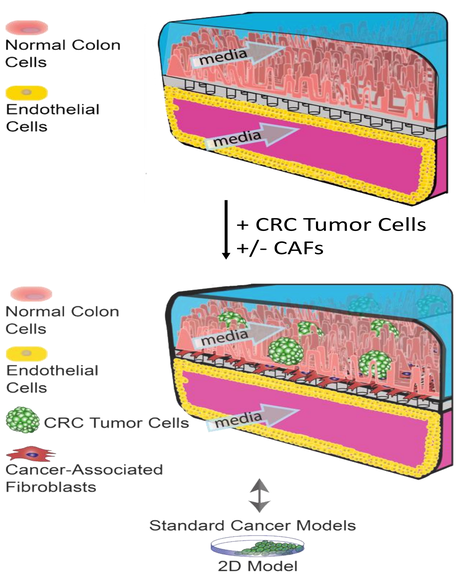
I. CRC-Tumor-on-Chip Development

Figure 1. CRC-Tumor-on-Chip



III. Impact of Tumor Microenvironment on CRC Cell Growth

A. 2D versus 3D Morphology

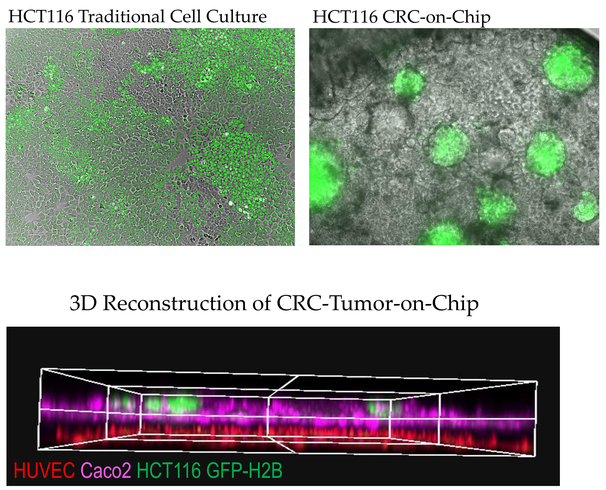


Figure 2. CRC Cells Grow Differently Depending on the Environment. HCT116 cells grow in 3D clusters on the chip compared to 2D monolayer on plastic.

B. 2D versus 3D Growth Rate +/- CAFs

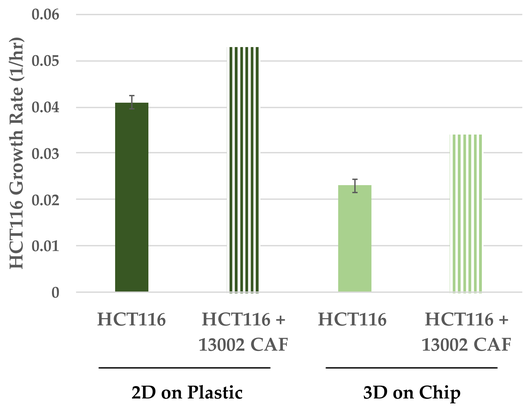


Figure 3. CRC Cell Growth Changes in Response to Environmental Cues. HCT116 cells grow 40% slower on the chip as compared to on plastic. HCT116s also grow faster in the presence of CAFs (30% on plastic, 50% on the chip).

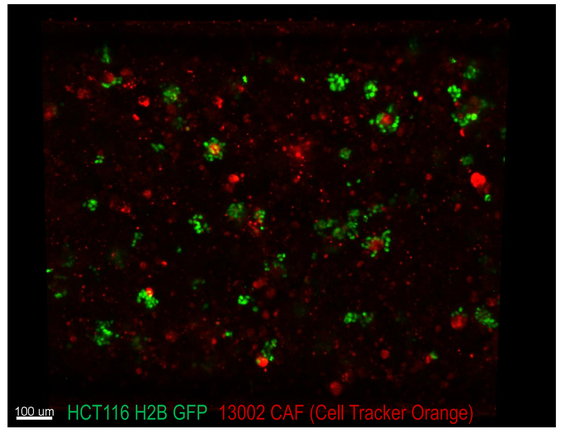


Figure 4. Patient Derived CAFs Grown on the CRC-Tumor-on-Chip. HCT116 cells co-cultured with 13002 CAFs at a 1:1 ratio.