Profiling Colorectal Cancer Cells & their Interactions with Fibroblasts in 3D Spheroid Cultures

Carnegie Mellon University Qatar

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ABSTRACT

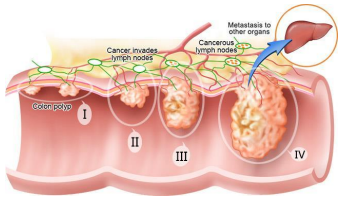
Colorectal cancer (CRC) is the third most common cancer and the second most fatal one as recorded by WHO in 2018. In Qatar, CRC is the most common cancer in men, and the second for women after breast cancer. With a relatively low survival rate (62-69%) and common risk factors, the need to understand the underlying metastasis mechanism and identify target effectors for therapy is essential to increase survival rate. Since fibroblasts are known to be the predominant cell population in a solid tumor microenvironment, the close interactions between fibroblasts and tumor cells can be a target mechanism to understand tumor progression and design therapeutic strategies. The ability of a tumor to metastasize is dependent upon the host tissue stroma that provides the necessary venue to invade through the blood stream or lymphatic pathway. Hence, the reprogramming of the naïve fibroblast to cancer associated fibroblasts (CAF) is a crucial step in cancer progression.

To map out the reprogramming mechanism and test potential therapies for colorectal cancer, a 3D model of the colorectal cancer will be developed and optimized to mimic the in-vivo microenvironment. Proliferation rate and morphological changes will be characterized through the process to determine the optimum seeding concentration and incubation for the model.

BACKGROUND

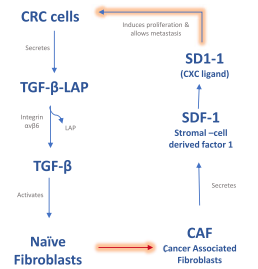
CRC Risk Factors

CRC Stages



Colorectal Cancer Stages Depicted (Chongco, 2019)

Suggested CRC-CAF bi-directional mechanism → promoting metastases
(Peng et.al, 2018)



RESULTS

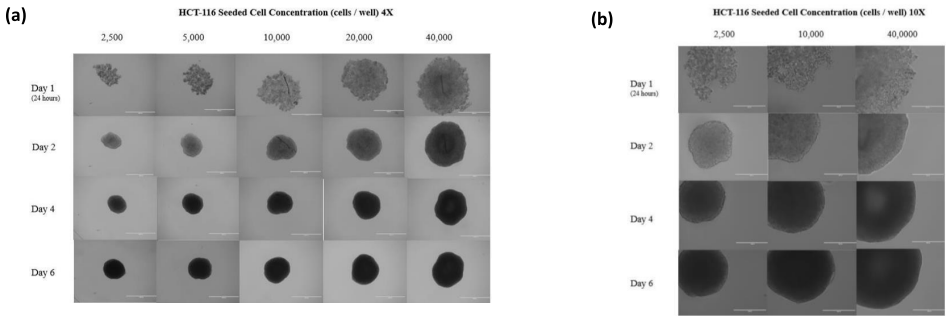


Figure 1 – HCT-116 3D Spheroids Formation & Development
(a) Images of spheroids at 4X magnification taken after 24 hours of seeding and at 48 hours intervals for a week to monitor spheroids' development. Scale bar: 1000 µm. **(b)** Representative images of the spheroids at a 10X magnification to assess the formation of the spheroids from aggregates. Scale bar: 1000 µm

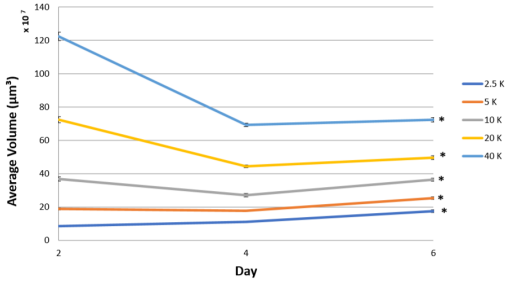


Figure 2 – Rate of HCT-116 cells proliferation in 3D spheroids.
Two diameter measurements of each spheroid image were determined using ImageJ Software from which the radius and volume of the spheroids were calculated. Volumes of spheroids are reported as mean + SD µm³; n = 8; *t-test $p < 0.05$; noting that all values plotted are significant.

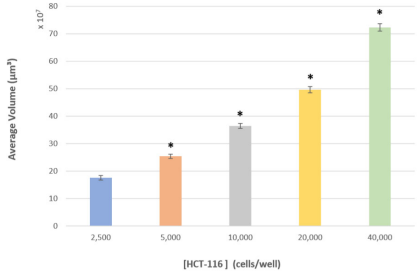


Figure 3 – Proliferation of HCT-116 cells based on cell concentration after 6 days of seeding. Two diameter measurements of each spheroid at all concentrations were measured after 6 days using ImageJ Software from which the radius and volume of the spheroids were calculated. Volumes of spheroids are reported as mean + SD µm³; n = 8; * t-test $p < 0.05$.

METHODS

3D Cell Culture. We used Nunclon™ Sphera™ ultra-low 96-well plate HCT-116 cells purchased from ATCC of the 4th passage were seeded at varying cell densities per well: 2,500, 5,000, 10,000, 20,000, and 40,000. The seeded cells were allowed to grow over a week in a humidified air incubator with 5% Co2/ 95% air at a temperature of 37°C. Imaging of all spheroids was done using EVOS FL Life Technologies Cell imager under normal light at two objectives : 4X and 10X.

Growth Curve. Using ImageJ Software the diameters of the imaged spheroids were measured. For each spheroid, a vertical and horizontal diameter was measured from which the radius and volumes of the spheroids were calculated using the following equation: vSphere = $4\,3 / \pi r^{-3}$. All cell densities seeded were done in octuplicates (n=8).

DISCUSSION

- As portrayed in Figure 1(a & b), HCT-116 cells did not form spheroids in the first 24 hours as expected, instead, the growing cell colonies aggregated together forming an irregular shaped .
- After 48 hours, the spheroids acquired a more regular shape, and became more compacted as cells proliferate within the encapsulated structure as shown in Figure 1(a).
  - We suggest that the HCT-116 cells began to secrete extracellular matrix (ECM) components 24 hours following seeding, enabling matrix modeling to occur, establishing stronger cell-cell and cell-ECM adhesion → spheroid formation and cellular proliferation → increased spheroid density overtime as the volume decreases (Figure 2).
- Figure 3 illustrates a positive correlation between initial seeding density and spheroid volume where the higher the seeded cellular density/well, the larger the formed spheroid is.

FUTURE DIRECTION

- Characterize HCT-116 –derived ECM markers to confirm the involvement of the stroma in the development and metastasis of CRC.
- Assessment of the expression of proliferation markers such as caspase 3 and proliferating cell nuclear antigen (PCNA) in HCT-116 3D spheroids.
- Optimizing the seeding time for the higher tested cell densities, 20,000 cells/well and 40,000 cells/well

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| Joana Khatib | Dr. Nesrine Affara | Department of Biological Sciences |