Characterization of Recruitment, Activation, and Induction of Naïve Fibroblasts in a Tumor-Educated Environment

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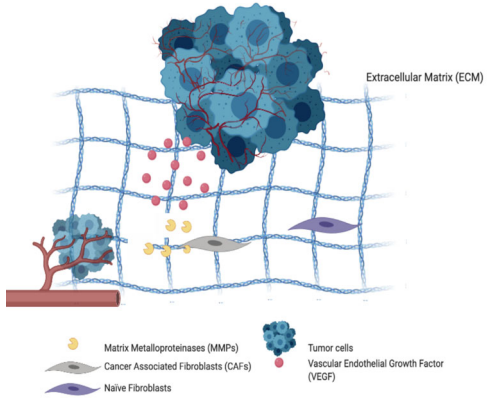
Introduction

Breast cancer is the most common cancer in women in Qatar. The role of the stroma surrounding the tumor can play a significant role in the tumor's progression, especially the extracellular matrix (ECM), which has fibroblasts that help in tumor progression, which can be activated by tumor cells. once activated they transform into cancer-associated fibroblasts (CAFs). Indeed, CAFs release proteins such as metalloproteinases (MMPs) that are present within the ECM, which enhance migration of tumor cells and trigger angiogenesis (as seen in the diagram on the left). This step will allow tumor form new blood vessels promoting tumor cell migration to distant organs. We used the fibroblast HFF cell line, that was derived from human foreskin and MCF-7 and T-47D cells that were derived from a breast cancer patient. Using 3D cell culture systems, which mimic the architecture of in vivo solid tumors, we will investigate the interaction between tumor cells and fibroblasts and whether we notice any changes in tumor cell's morphology or behavior when in contact with the fibroblasts.



Methods

3D cell cultures. MCF7 and T-47D grown were growing in incubation at 37˚C replenished with 10 % FBS of DMEM Cell Culture Media in T75 flasks, each well had 200 μl of sample. 5% of FBS in DMEM Cell Culture Media was used to replenish the 3D cells.

Migration Assays. Boyden chambers of 8 well plate 3mm deep were used to plate *105* cells per well of fibroblasts strain (HFF) and tumor strains (MCF7 and T47D). The plates were incubated at 37˚C for 24 hours. After 24 hours of incubation the cells were stained using H&E staining.

Western blot analysis. Protein samples after tissue lysates were collected and loaded on SDS-PAGE gels and transferred on to membranes. Using β-actin antibodies as primary antibody and Horse radish Peroxidase as secondary antibody.

Results & Discussion

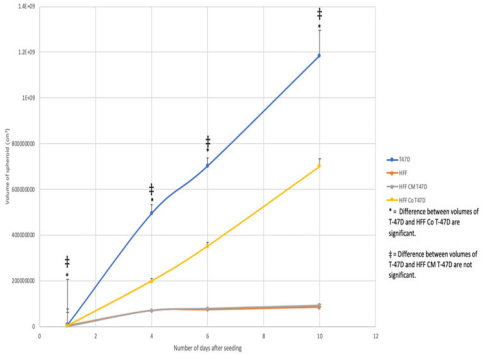


Figure 1. Spheroid Volume of HFF interplay with T-47D in 3D cultures. 10,000 cells (but 5000 cells for each of the co-cultures) were seeded.

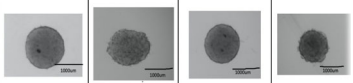
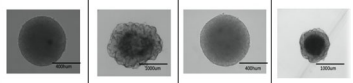
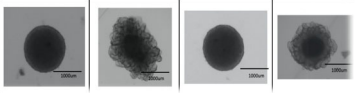
Amount of time after seeding	HFF	T-47D	HFF CM T-47D	HFF Co T-47D
24 hours				
4 days				
7 days				

Table 1. Morphology of HFF interplay with T-47D in 3D cultures from day 1 - 7

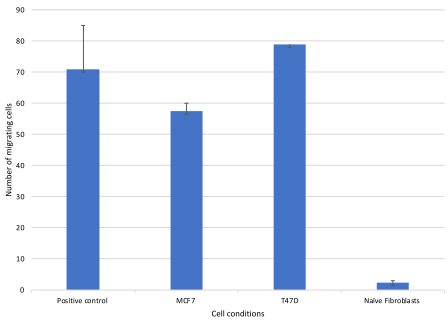


Figure 2. Number of migrating HFF cells across Boyden Chambers to tumor-educated media. Migration assay is shown above using an 8-well plate to plate each cell condition in duplicates and incubated at 37˚C. H&E staining was used to count the fibroblasts.

3D cell culture assays of T-47D and HFF cells. As seen in figure 1 the HFF + T-47D cells grew in volume across time compared to the HFF + T-47D CM only. This can be proven also by the morphology of the cells shown in table 1, they grew in circumference as well. This illustrates that cell-cell interaction is required for proliferation and not only tumor microenvironment.

Boyden chamber Migrations of HFF cells. As illustrated on figure 2 the fibroblasts migrated across the membrane to a richer environment in the bottom well in the positive control. We can also see significance in the HFF migrating to MCF7 CM, this can explain how tumor cells effect on the migratory behavior of fibroblasts in *vivo*. Factors released by the tumor can mediate migration for the fibroblasts. Furthermore, as shown in naïve fibroblasts, no HFF migrated across to a neutral environment.

Future work

Additional work involves assessing the activation of fibroblasts using using additional cell lines such as Human umbilical vein endothelial cells (HUVECs). Angiogenesis involves the formation of blood vessels. Hence, we would see how do tumor growth factors in *vivo* recruit endothelial cells to induce angiogenesis. To quantify MMPs in the future we will use the 3D cell cultures of HFF with T-47D cells and HFF alone to compare the expression in both. We will evaluate this by using western blot analysis and probing for MMP proteins using specific antibodies.

References

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