CANCER ORGANOTROPISM-ON-A-CHIP

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ABSTRACT

Cancers exhibit organ-specific metastasis (i.e. organotropism) and studying the underlying mechanism remains challenging due to limitations in co-culture models. In this work, we report an organ-on-a-chip device capable of co-culturing cancer spheroids with up to four relevant tumor microenvironment (TME) stromal cell types. We utilized this device for characterizing the migration pattern and secretomic crosstalk between stromal cells and spheroids of breast cancer cells with known and distinct organotropism.

KEYWORDS: organ-on-a-chip, secretomic profiling, metastatic organotropism, tumor microenvironment

INTRODUCTION

Decoding the complex biological network within the TME, which consists of spatiotemporally-regulated cell-cell and cell-matrix interactions, is crucial in advancing our understanding of tumor progression and metastasis [1]. Furthermore, the TME secretome remains a key determinant in metastatic organotropism by priming the pre-metastatic niche via signaling proteins and extracellular vesicles (EVs) [1, 2]. Thus, an intricate 3D co-culturing system enabling precise tissue localization of multiple cell types while allowing discrete sampling without disturbing the system's biochemical gradients is needed to recapitulate and analyze the dynamic interactions within the TME.

EXPERIMENTAL

We developed an organ-on-a-chip device for co-culturing cancer spheroids with up to four relevant TME stromal cell types. Spheroids of MDA-MB-231 triple-negative breast cancer cell line, or its brain-, bone-, lung-, and liver-organotropic subpopulations were seeded into the spheroid chamber along with organ-native stromal cell types in side chambers surrounding the spheroid, mimicking cancer-stromal configurations observed *in vivo*. Conditioned media from chamber-specific open wells was sampled and profiled for secreted proteins and EVs throughout the experimental time-course using antibody microarrays.

RESULTS AND DISCUSSION

Our initial design consists of a spheroid chamber and two side chambers, as shown in **Figure 1A** and **1B**. Cancer spheroid and up to two stromal cell types were seeded into individual chambers and cultured for up to 1 week, as shown in **Figure 1C**. When co-culturing brain-tropic breast cancer spheroid with HBEC brain endothelial cell line and IMR-90 lung fibroblast cell line, we successfully initiated fibroblast migration on-chip, with fibroblast sprouts detected as early as day 1 of co-culture, as shown in **Figure 2A - D**. We then co-cultured breast cancer spheroids of various organotropisms with the same co-culture setup. Our preliminary microscopy data suggested that migration patterns of the fibroblast and cancer spheroids varied depending on spheroid organotropism, as shown in **Figure 2E - G**. Interestingly, we observed the most fibroblast sprouting in brain-tropic spheroid, hinting at the potential synergistic effects of matched endothelial cell type and organotropism. Meanwhile, the expression of matrix metalloproteinase-3 (MMP-3) and EV populations in our system were measured, as shown in **Figure 3**. When sampled at day 3 (**Figure 3A**) and day 6 (**Figure 3B**), we observed that while the majority of matrix-degrading MMP3 expression was found in the fibroblast chamber in most devices, levels among the different organotropism differed between the two time

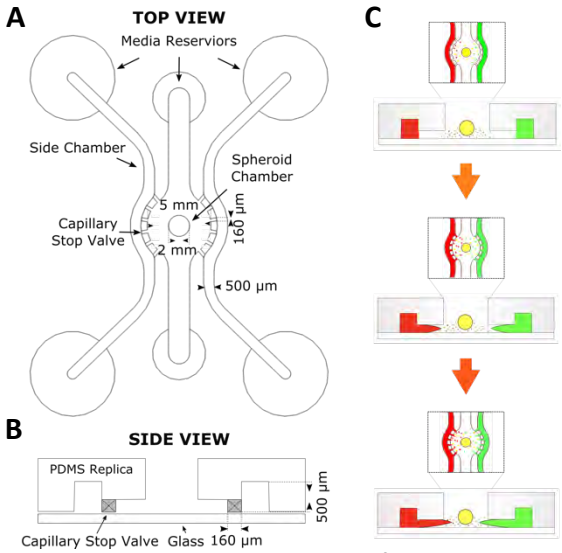

***Figure 1:** (A) Top view and (B) side view of co-culture device. (C) Co-culture workflow for mimicking the in vivo cancer-stromal configurations.*

points. We also observed heightened CD63 and CD9 EV expression (**Figure 3C - D**) in the fibroblast chamber when co-cultured with parental and bone-tropic spheroids. Furthermore, we observed heightened CD9 EV expression (**Figure 3D**) in the brain endothelial chamber only when co-cultured with brain-tropic spheroid, hinting at potential brain endothelial activation by brain-tropic spheroid. Motivated by our results, we further developed a next generation design capable of co-culturing up to four stromal cell types, as shown in **Figure 4A** and **4B**. Ongoing work involves the modeling of organotropic spheroids with organ-native fibroblasts, endothelial cells, and immune cells to further mimic the *in vivo* cancer-stromal configurations.

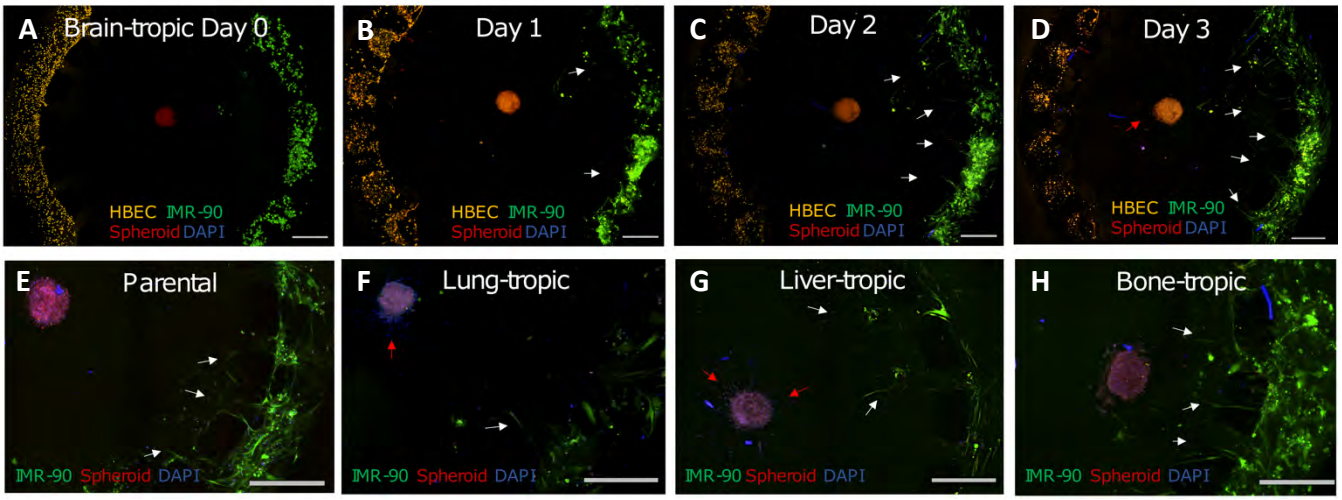

Figure 2: *(A-D) Time-course confocal images of brain-tropic breast cancer spheroid co-cultured with brain endothelial cell HBEC and lung fibroblast IMR-90.* ***(E-H)*** *Confocal images of parental breast cancer* ***(E)*** *and its organotropic subline* ***(F-H)*** *spheroids co-cultured with the same setup at day 2. White and red arrows indicate directional migration of fibroblast and spheroid, respectively. Scale bar = 500 µm.*

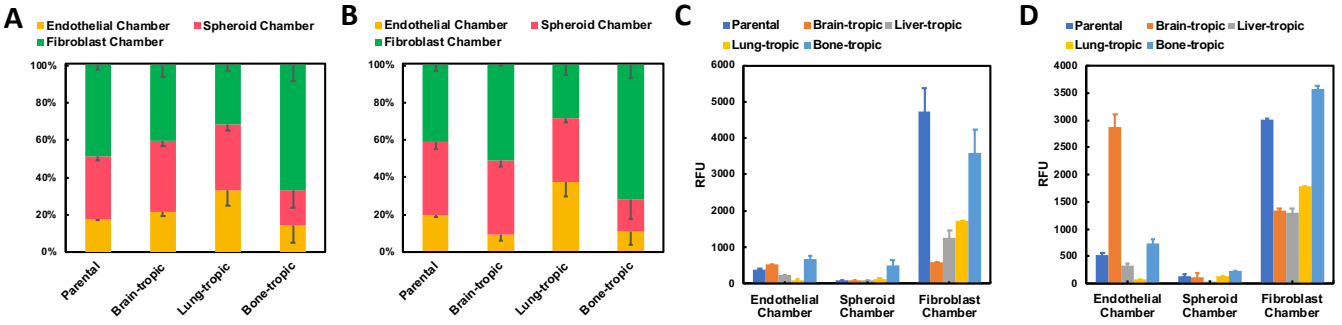

Figure 3: *(A-B) Ratio of MMP3 expression between the co-culture chambers within device seeded with parental and its organotropic sublines at day 3 (A) and day 6 (B).* ***(C-D)*** *EV expression of CD9 (C) and CD63 (D) between the co-culture chambers within device seeded with parental and its organotropic sublines at day 2 of co-culture.*

CONCLUSION

We believe our platform opens up potential avenues for bridging our understanding of the highly dynamic TME secretome and its heterogenous effects on tumor-stromal migration and metastatic organotropism.

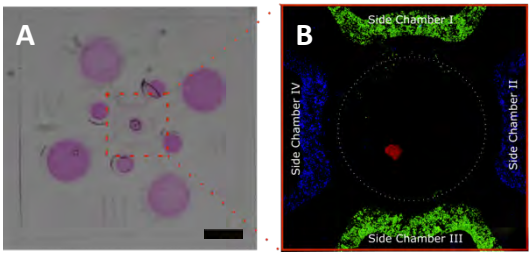

Figure 4: *(A) Brightfield and (B) Confocal image of On-chip co-cultured breast cancer spheroid and 4 side chancel cell types. Scale bar = 5 mm.*

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