Microengineered Tumor-On-Chip Model To Decipher The Role Of Stromal Crosstalk On Tumor Progression

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INTRODUCTION: Breast cancer is one of the leading causes of mortality among women worldwide. It is now well accepted that tumors are not just a mass of proliferating cells rather a heterogenous disease with complex tumor microenvironment (TME) and multitude of cells. Multidimensional interaction of cancer cells with various non-cancerous supporting stromal cells such as fibroblasts and immune cells within the TME aids in generating an ecosystem that leads to acquiring various hall marks of cancer such as tumor progression, immune evasion, and therapeutic resistance. To fully understand the continual tumor-stroma crosstalk, there is a critical need for better *in vitro* models that mimics the heterogenous complexity of the TME. This study focuses on understanding the synergistic effect of two prominent stromal population namely cancer associated fibroblast (CAF) and macrophages (Mϕ) on tumor progression by developing a tri-culture on-chip microfluidic TME model. The reported 3D microfluidic model enables spatial arrangement of tumor cells and stromal cells for tri-culture assay in a well-controlled and organotypic manner.

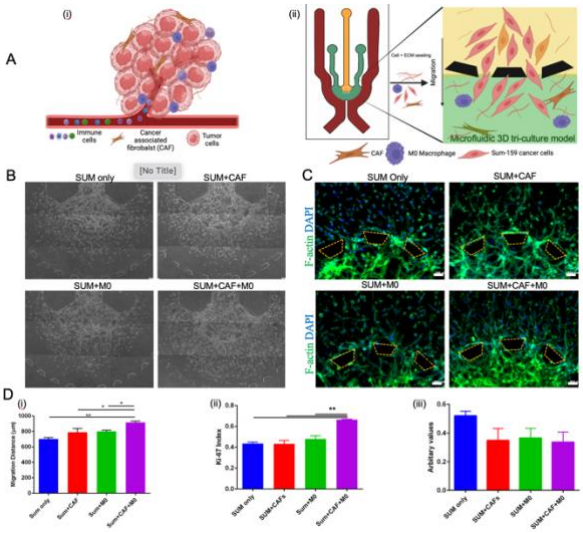

Figure 1: A) (i) Schematic representation of tumor microenvironment (TME) (ii) 3D tri-culture microfluidic model. B) Brightfield images of cells cultured in various culture conditions. C) Fluorescent images of cytoskeletal stained cells in the tumor-stromal front. D) Assessment of migration, proliferation, and morphometric changes of cells within the tumor-on-chip devices. Data are presented as Mean ± SEM. Significance was calculated using one way-ANOVA; * indicates p value <0.05, ** p-value <0.01. Trapezoids (dashed and black) represents the posts between the tumor and stromal region. Created with BioRender.com

MATERIALS AND METHODS: The tri-culture system was established in the microfluidic model that was previously developed in our laboratory. The microengineered platform allows the juxta position of tumor and stromal region with distinct ECM. To understand the combinatorial influence of stromal population on tumor invasion, triple negative breast cancer (TNBC) cell line- SUM159 cells were tri-cultured with patient derived CAFs and THP-1 derived naïve Mϕ, generated by treating THP-1 cells with Phorbol 12-myristate 13-acetate. SUM-159 mixed with collagen and Matrigel® forms the tumor region while CAFs with naïve Mϕ embedded in collagen forms the stromal region **(Fig 1A).**

RESULTS AND DISCUSSION: The synergistic influence of CAF+Mϕ on tumor invasion within our 3D microfluidic TME model was pronounced as opposed to monoculture (SUM) and co-culture (SUM+CAF, SUM+Mϕ) groups. Specifically, SUM cells in the tumor region were allowed to migrate to the stromal region within the 3D scaffold for a period of 3 days and the invasive front of tumor cells were measured. Significant increase in migration distance of SUM cells was observed in tri-culture condition as compared to mono-culture condition **(Fig 1B, D(i))**. Also, we observed significant difference in ki-67 expression in tri-culture condition in comparison with monoculture crosstalk and co-culture conditions **(Fig1 D(ii)).** Next, we performed morphometric analysis of tumor cells in mono-, co- and tri-culture conditions by staining the cells for F-actin fibers **(Fig 1C)**. Quantification of morphometric parameters showed a trend in circularity of the cells in the presence and absence of stromal component **(Fig 1D(iii))**. But no significant difference was observed between culture groups.

CONCLUSION: We established a tri-culture 3D microfluidic tumor-on-a-chip system that recapitulates the complexity of the native TME by incorporating stromal and immune cells. To further our understanding from the molecular perspective, our future studies will be focusing on understanding the fate of CAFs and Mϕ on transcriptomics of the tumor cells to unveil the signaling pathways that are involved in cancer progression. We believe that understanding the molecular and cellular basis from the incorporation of various stromal cells will help in development of drug candidates that can target resistance pathways and non-cancerous cellular population.

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