Comparative Analysis of the Role of the Tumor Cells in Educating Naïve Fibroblasts into Cancer-Associated Fibroblasts Using 2D vs. 3D Environments

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Abstract

Activated fibroblasts, also known as Cancer-Associated Fibroblasts (CAFs), comprise a population of stromal cells within the heterogenous tumor microenvironment that promote cancer growth by inducing extracellular matrix remodeling, angiogenesis, recruitment of immune cells, and migration of tumor cells. Transition of local or recruited naïve fibroblasts (NFs) into CAFs is triggered by the crosstalk between the fibroblasts and the tumor cells, resulting in the upregulation of specific markers, including α-SMA, FAP, vimentin, and PDGFR-α, which have been used to identify and study the various CAF populations. However, marker variation within CAF populations is extensive due to their heterogeneity and high plasticity, making marker-based identification of CAF unreliable and contradicting between subpopulations at times. Furthermore, although reprogramming of fibroblasts to CAFs via interactions with the tumor cells has been recapitulated in vitro using regular 2D cultures, it has yet to be evaluated in 3D cultures that mimic in vivo solid tumors to find out possible contextual differences. The current research aims to study the effect of the human breast cancer cell line (T47D cells) on reprogramming of Human Skin Fibroblasts (HFF) into CAFs by comparing expression of CAF markers using 2D and 3D culture models. CAF activation was evaluated using 2 different approaches: a- Indirect co-culturing where HFFs are cultured in conditioned medium collected from tumor culture assessing the role of tumor cell-derived factors. b- Direct co-culturing where HFFs and tumor cells are grown together. Results from the current studies will add more clarity to the growing field of CAFs and provide a model system to further assess the different functions of CAFs.

Introduction

Cancer is not a disease caused solely by an isolated tumor cell population composed of malignant neoplastic cells, but rather a condition arising from and sustained by the collaboration of various cell types residing in the tumor cellular environment (TME). TME is a highly heterogeneous and dynamic network of multiple local or recruited stromal cell types: adaptive and innate immune cells, fibroblasts, vascular endothelial cells and the Extracellular Matrix (ECM). The initially tumor-suppressive stromal tissue evolves into this tumorigenic niche upon modulatory cross-talk with the tumor.

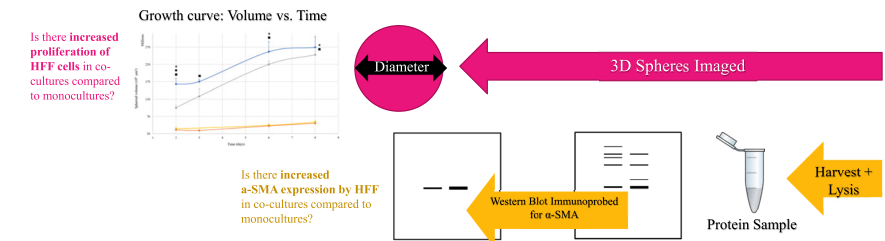
Cancer-Associated Fibroblasts (CAFs) are one of the dominant cell types in the TME that arise from fibroblasts of miscellaneous origins including the resident naive fibroblasts. Naïve fibroblasts are cells with negligible metabolic and expression activity that are mainly responsible for secreting ECM molecules. Upon education by the tumor cells, naïve fibroblasts acquire extreme myofibroblastic phenotypes such as enhanced secretion of growth factors and cytokines and overexpression of Alpha-Smooth Muscle A (α-SMA), which is used extensively as a CAF-marker in biopsies and research to detect and study CAFs. CAFs are documented to be markers of poor prognosis and as such, studying the Naïve-to-CAF activation is important to understand cancer development, has prognostic relevance and potential as a target for anti-cancer treatments.

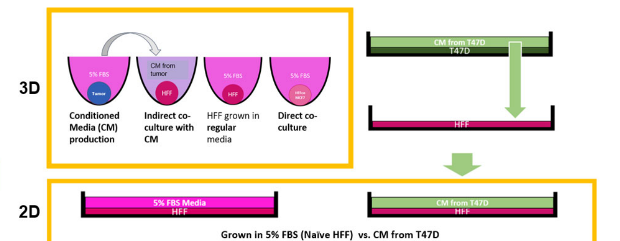
Both direct and indirect mode of the tumor-fibroblast education has been documented in research with direct and indirect co-culturing in vivo models.

It is important to compare this relationship in 2D and 3D models as they these two conformations are known to result in different cell characteristics

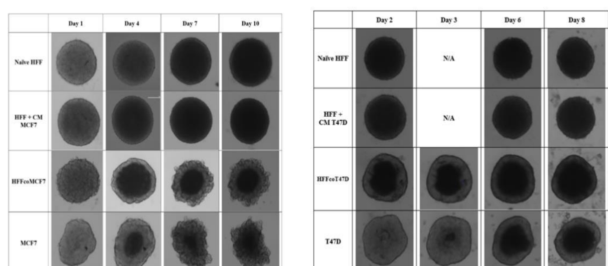
Our hypothesis was that the indirect co-culturing of 2D and 3D Human Foreskin Fibroblasts (HFF) with tumor cells from MCF7 and T47D will result in their CAF-activation, marked by an increase in α-SMA expression and bigger 3D spheres due to increased proliferation rate.

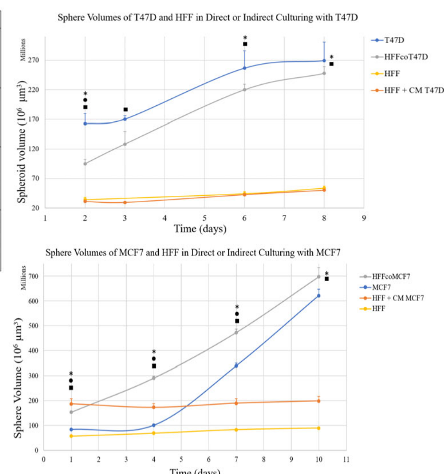
Methodology





Results





How much of direct co-culture growth attributable to HFF and how much to MCF7 or T47D?

→ Must be able to differentiate between cell lines in the future

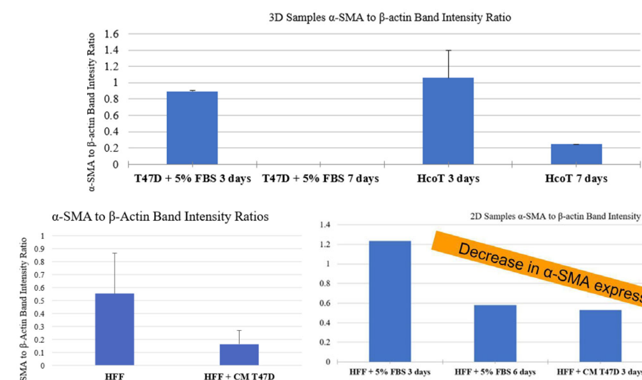
A highly proliferative core in the direct co-cultures compared to monocultures.

Seeding amount mistake for HFF or HFF + CM MCF7, interpreted to have similar sphere volumes and growth rates.

MCF7-HFF direct co-culture > MCF7 > HFF = HFF + CM MCF7

T47D > T47D-HFF direct co-culture > HFF = HFF + CM T47D

No difference between HFF and HFF + CM T47D, no proliferative effect seen due to indirect co-culturing.



No statistically significant difference between T47D and HcoT.

HFF = HFF + CM T47D

No CAF-activation or a-SMA not viable as the only CAF-marker

A decrease instead of an increase Could it be that HFF cells are simply dying?

Conclusion

No increase in the expression of a-SMA could was observed in any of the 2D or 3D, direct or indirect co-culture models with breast cancer cell lines (BCC) MCF7 or T47D. Hence, we conclude that there was either no CAF-activation of HFF along with the possibility that a-SMA is not reliable as a CAF-marker by itself, For future directions, focus will be on the direct co-culture models since an accurate model includes HFF and tumor cells together followed by CAF-tumor cross-talk. Instead of relying only on a-SMA, a wide array of CAF-markers which include Vimentin, Platelet Derived Growth Factor Receptor-Alpha (PDGFR-a) and Fibroblast Activating Protein (FAP) to get a more detailed picture of the fibroblast expression profile before and after education, to make more accurate interepretations. FAP will be especially useful since it is specific for fibroblasts and do not get expressed in the epithelial BCCs MCF7 and T47D.

The morphological studies are insufficient as they are for reliable interpretation of CAF-activity as the 3D sphere content cannot be analyzed by external observation. To overcome this, the cell lines can be transfected with different fluorescent proteins. This also enables cell type-based sorting of co-culture cell populations based on different fluorescent properties.

Incorporating other assays that assess the presence of CAF by measuring CAF-specific characteristics will be very valuable for the reliability of research conclusions. Scratch assays or matrigel migration chambers can be used to assess increased proliferation and motility of tumor cells upon cross-talk with CAFs and fibroblasts can be stained for PCNA to assess their increased proliferation upon CAF-activation. Finally, externally acquiring CAFs to use as positive controls in CAF-marker expression studies would be very useful.

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