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Screening the Effect of Faradarmani Consciousness Field on *Ex vivo* Controlled Microenvironment on Solid 4T1 Tumors

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Abstract

Personalized cancer medicine is currently focused on knowledge of the cancer mutation repertoire and the tailored application of drugs that targets altered genes or pathways in individual patients. Thus, a critical need exists for more sophisticated *ex vivo* diagnostic methods that recapitulate human tumor biology and predict response to the targeted and immune-based therapies in real-time. T-Consciousness Fields are immaterial, and non-energy Fields, and their influence on the matter and energy has been observed in several biological and physical studies. In the present study, we developed a 3D Microfluidic controlled microenvironment device that consists of two media channels parallelly running. They are located on either side of an extended, central region, containing the tumor cell spheroids embedded within an extracellular matrix. In these conditions, we investigated the influence of Faradarmani Consciousness Field on this 4T1 cell line. According to the results, under the influence of the treatment of Faradarmani CF, the 4T1 cell line showed a significant increase in survival compared to the control groups.

Keywords: *ex vivo*, Microfluidic, Cell line, Cancer, T-Consciousness Fields, Faradarmani Consciousness Field

Introduction

Cancer is a prominent cause of death worldwide, accounting for nearly 10 million deaths in 2020 (Sung et al., 2021). The risk factors like genetic background, environmental stresses, and improper diet generally lead to molecular changes or mutations which cause the initiation of carcinogenesis (Padma, 2015). In malignant tumors, metastasis poses the biggest problem for cancer treatment. It has been hypothesized that the epithelial to mesenchymal transition (EMT) is an important event in the metastatic cascade. EMT is involved in the generation of tumor cells with stem cell properties and resistance to certain therapeutic interventions (Bullock et al., 2012; Nieto et al., 2016; Lambert et al., 2017). Therefore, it has been considered a viable therapeutic target to combat metastatic disease (Kalluri and Neilson, 2003). Spotting a primary tumor before metastasis is crucial. However, physical examination and traditional imaging methods like MRI, PET, CT, and X-ray have a detection limit for smaller metastasis (Van Dalum et al., 2012). In a patient with solid tumors, Circulating Tumor Cells (CTCs) can be found in the blood, so biomarkers may provide a way to monitor disease progression more directly than traditional imaging methods (Van Dalum et al., 2012, Yang et al., 2019). CTCs hold information about a tumor that could be a key to the cancer diagnosis or treatment (Williams, 2013).

Chemotherapy as an important treatment for cancer patients has some limitations, including a lack of selectivity for tumor cells over normal cells, systemic toxicity, and the appearance of drug-resistant tumor cells (Xu and McLeod, 2001). Numerous therapeutic strategies have been focused on drugs that target altered genes or pathways in individual patients. For example, *Kristen Rat Sarcoma viral oncogene* (KRAS) is one of the earliest oncogenic drivers, so inhibition of KRAS may be a viable therapeutic strategy for this disease (Muzumdar et al., 2017, Uras et al., 2020). A variety of *in vitro* approaches have been used in cancer biology and drug discovery. However, *in vitro* models do not entirely reproduce the cellular diversity and complexity of interactions in tumor niches (Bellido et al., 2020). Two-dimensional (2D) cell culture has many limitations, such as disturbance of interactions between the cellular and extracellular environments, changes in cell morphology, polarity, and method of division (Kapałczyńska et al., 2018). It has been reported that around 95% of new anticancer drugs eventually fail in clinical trials, despite robust indications of activity in existing *in vitro* pre-clinical models (Hickman et al., 2014).

Recent advances in the microfluidic cell or tissue culture technology can be used as a novel *in vitro* drug screening method with high-throughput applications (Tsui et al., 2013) and the development of "organs on chips", enable experimentalists to recapitulate the multicellular architecture, tissue-tissue interfaces and the physiologically relevant physical microenvironment of cancers growing within living human organs while sustaining vascular perfusion *in vitro* (Sontheimer-Phelps et al., 2019). Using 3D culture, the inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit was investigated and identified concurrent inhibition of the TBK1/IKKε, Janus-activated kinase (JAK), and MEK signaling as an effective method to inhibit the actions of oncogenic KRAS (Zhu et al., 2014). A study, screening the therapeutic EMT blocking agents using 3D microfluidic systems, showed that there are significant differences in response to drugs between 2D and 3D and between monoculture and co-culture (Aref et al., 2013). In addition, Jenkins et al. and Aref et al. evaluated tumor-immune interaction in 3D culture using patient- or murine-derived organotypic tumor spheroids (MDOTS/PDOTS) (Jenkins et al., 2017; Aref et al., 2018). Their research showed that this platform can model response to the PD-1 blockade and facilitate precision immune-oncology efforts and ultimately personalized immunotherapy.

In this study, the effects of Faradarmani Consciousness Field on changes in 4T1 cell line was evaluated in *ex vivo* conditions. This field possesses a non-frequency nature. Therefore, it is not possible to measure it with quantitative instruments, but its effects can be recorded through designing experiments (Taheri, 2013). The influence of this field is initiated with a short attention to the subject under study. Previously, the effects of Faradarmani

Consciousness Field on MCF-7 cell line (Taheri et al., 2020), and the mice 4T1 breast cancer model were investigated (Taheri et al., 2022). The obtained results showed that although Faradarmani induced replication and survival of MCF-7 cell line, inhibited the growth of tumor cells and metastasis in 4T1 animal model. The current experiment with three-dimensional culture allows the reproducibility of previous experiment.

Methods and Materials

Application of Faradarmani CF

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.cosmointel.com). More details are presented in the general considerations of this issue.

2D cell culture

The 4T1 cell line was cultured in a humidified atmosphere with 5% carbon dioxide at 37°C in Dulbecco modified Eagle medium/Nutrient Mixture F-12 Ham supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin solution.

Spheroid preparation

The adherent cells were grown to 80% confluence. Monolayers were rinsed twice with PBS. After draining well, 2 mL (for 100 mm plates) of 0.05% trypsin-1 mM EDTA was added and incubated at 37°C until cells detach. Next, the detached cells were transferred to a 15 mL conical tube and centrifuged at 1200 rpm for 5 mins. Then, the cell pellet was resuspended in a 2 mL complete culture medium and the cells were counted. Using a pipette, 20 µL of cells were deposited on an inverted lid from a 100 mm tissue culture dish. The lid containing 45 deposited cells was inverted in the PBS-filled chamber at 37°C under 5% CO_2 and 95% humidity and was monitored and incubated until the aggregates were formed. After 24h of incubation, drops were washed by the medium from the lid and centrifuged in a conical tube at 1200 rpm for 5 mins to obtain a spheroid pellet.

3D cell culture (day 0)

In order to achieve the proper fractions (40-100 µm), spheroids were filtered by 100 µm and 40 µm cell strainers respectively, and centrifuged at 1200 rpm for 5 min. Subsequently, cell pellets were resuspended in collagen hydrogels (type I rat tail collagen 2.5mg/ml, Corning Co.). Hydrogels containing spheroids were injected into the central channels of 3 (A, B, C) microfluidic devices and incubated for 30 min at 37 °C in humidified chambers. Microfluidic chips were designed by and manufactured at AIM BIOTECH (DAX-1, AIM BIOTECH, https://www.aimbiotech.com/). We used 3 devices for each sample as three replications for the experiments (3 for A, 3 for B, and, 3 for C). Hydrogels were hydrated with DMEM/F12 + 10% FBS + 1% pen/strep and incubated at 37°C for 24 hours.

Treatment of the spheroids (day 0 to day 3)

A (control: DMEM + FBS + pen/strep), B (treatment), and C (control: doxorubicin) were placed in different places in the incubator.

Live /dead assay

Spheroid viability was evaluated on day 3 after staining with AO/PI using the ViaStain™ AO/PI Staining Solution (Nexcelom, CS2-0106) according to the manufacturer's protocol.

Imaging of spheroids and data analysis

Spheroids were imaged on an inverted Nikon Eclipse Ti microscope equipped with a Nikon DS-Qi1Mc camera using NIS-Elements software. The total area of Acridine orange-stained live (green) cells versus Propidium iodide-stained dead (red) cells was quantified.

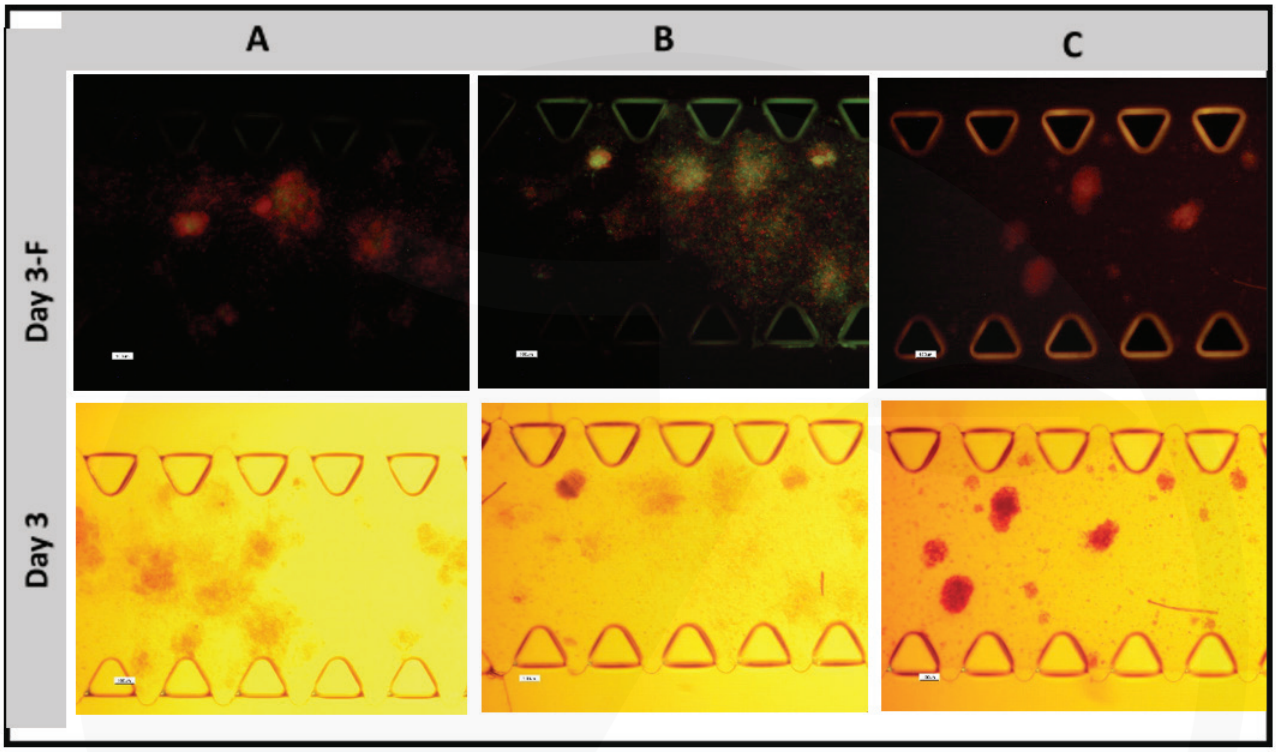
Statistical analysis

In this study there were three replicates in each group and no data were excluded. Data were statistically analyzed using analysis of variance one-way (ANOVA) with GraphPad software (version 9), and a confidence level of 95% considered as significant threshold of data.

Results and Conclusion

To evaluate the effects of the treatment on 4T1 spheroids in microfluidic devices, live and dead cells were counted in fluorescent images from cells in groups A (DMEM-treated cells), B (treated cells), and C (Doxorubicin-treated cells as positive control) on day 3 of the study. The results showed that the percentage of live cells in group B was significantly more than in groups A and C. In other words, treatment slightly improved the viability of the cells. Figure 1 presents the images and the graph.

A)



B)

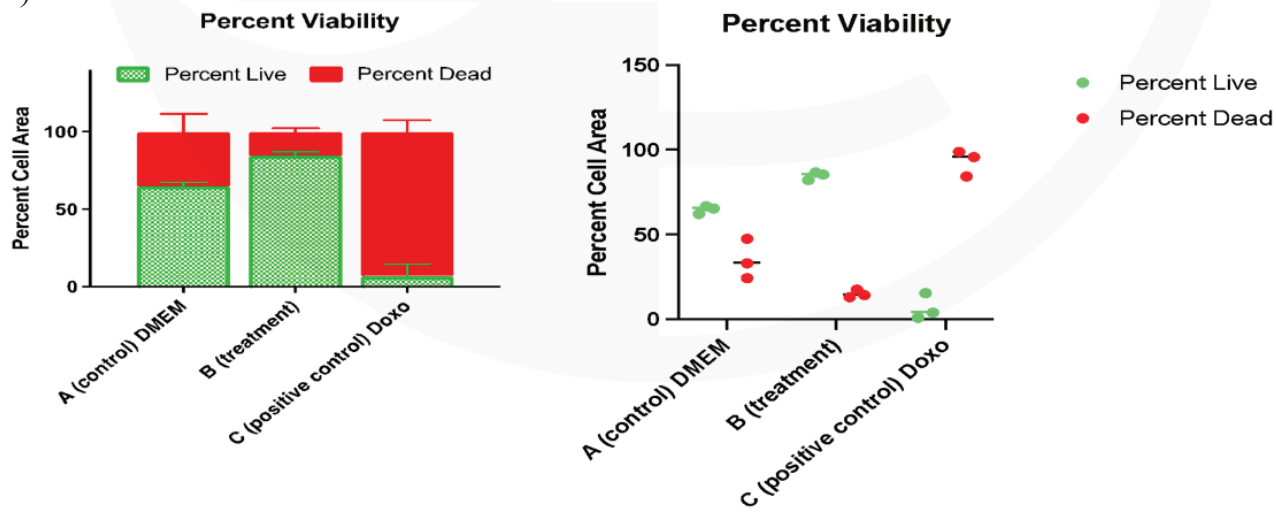


Figure 1. Percentage of cell viability on day 3 of the study. A) Optical and fluorescent Images from the spheroids on day 3. Green: live. Red: dead. Scale bars: 100µm. A: DMEM-treated cells (as control), B: Treated cells, C: Doxorubicin-treated cells (as positive control).

The obtained results in this study were in accordance with observations made on the *in vitro* MCF7 culture (Taheri et al., 2020). Similarly, in the current work, a significant enhancement in survival was observed in Faradarmani-treated 4T1 cell line compared to the control. According to Taheri, Faradarmani CF can transmit information to optimize the system under the study. Considering independent nature of cancerous cell lines in the body of living organisms, it seems that as observed with MCF7 cells, this cell line demonstrates increased proliferation outside the body when exposed to Faradarmani Consciousness Field. Further research on the primary cancer cells (patient), and also study the molecular and immunological changes in variety of cell lines under the influence of Faradarmani Consciousness Field are on the agenda of the authors of this study.

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