Screening the Effect of Faradarmani Consciousness Field on the 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. Taheri Consciousness Fields are immaterial, and non-energetic fields and their influence on the world of 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 (CF) on this 4T1 cell line. According to the results, under the influence of the Faradarmani CF treatment, the 4T1 cell line showed a significant increase in survival compared with the control groups.

Keywords: ex vivo, microfluidi , cell line, cancer, Taheri 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", enables experimentalists to recapitulate the multicellular architecture, tissue-tissue interfaces and the physiologically relevant physical microenvironment of cancers growing within living human organ 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., 2017 and Aref et al., 2018 evaluated tumor-immune interaction in 3D culture using patient- or murine-derived organotypic tumor spheroids (MDOTS/PDOTS). Their research showed that this platform can model response to the PD-1 blockade and facilitate precision immune-oncology efforts and ultimately personalized immunotherapy.

The nature of consciousness and its place in science has received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the1980s, Mohammad Ali Taheri introduced novel fields with a non-material/non-energetic nature named Taheri-Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. According to this theory, there are various TCFs with different functions, which are the subcategories of a networked universal internet called the Cosmic Consciousness Network (CCN). The major difference between the theory of TCFs and other theoretical concepts about consciousness is related to the practical application of the TCFs. TCFs can be applied to all living and non-living creatures, including plants, animals, microorganisms, materials, etc.

Mohammad Ali Taheri, the founder of Erfan Keyhani Halqeh, a school of thought, introduced a new science in 2020 as a branch of this school. He coined the term Sciencefact for this new science because it utilizes scientific investigations to prove the existence of T-Consciousness as an irrefutable phenomenon and a fact. Although science focuses solely on the study of matter and energy and Sciencefact, by contrast, explores the effects of the [non-material/non-energetic] TCFs, Sciencefact has provided a common ground between the two by conducting reproducible laboratory experiments in various scientific fields, and it has used the scientific approach in proving TCFs.

The influence of the TCFs begins with the Connection between CCN as the Whole Taheri Consciousness of the universe and the subjects of study as a part. This Connection called "Ettesal" is established by a Faradarmangar's mind (a certified and trained individual who has been entrusted with the TCFs). The human mind has an intermediary role (Announcer) which plays a part by fleeting attention to the subject of study and then the main achievement obtained as a result of the effects of the TCFs. These fields cannot be directly measured by science, but it is possible to investigate their effects on various subjects through reproducible laboratory experiments (Taheri 2013).

The research methodology in the study of T-Consciousness has been founded on the process of Assumption, Argument, and Proof, in which the basic Assumption is: The Cosmos was formed by a third element called T-Consciousness that is different from matter and energy.

The Argument: The existence of TCFs can be demonstrated by its effects on matter and energy (e.g., humans, animals, plants, microorganisms, cells, materials, etc.)

The Proof: is the scientific verification of the effects of TCFs on matter and energy (according to the Argument) through various reproducible scientific experiments.

Accordingly, to investigate and verify the existence, effects, and mechanisms of TCFs, the following five research phases (Phases 0 through 4), and the aims of each phase are outlined below.

Phase-0 studies aim to prove the existence of TCFs by observing their effects. The nature of T-Consciousness and what it is will not be addressed in this phase. Phase-1 explores the varied effects of different TCFs. Phase-2 examines the reason behind the varied effects of these fields. Phase-3 investigates the mechanism of TCFs effects on matter and energy. Finally, Phase-4 draws significant conclusions, particularly with regard to the mind and memory of matter and their relation to the T-Consciousness, etc.

Previously, in two experiments, the effects of Faradarmani CF on the survival and death of the MCF-7 breast cancer, and the mice 4T1 breast cancer model were investigated. Obtained results demonstrated that Faradarmani CF induced cell proliferation and survival in MCF-7 (Taheri et al., 2020a), and in mice, 4T1 model Faradarmani CF inhibited the growth of cancerous masses and metastasis (Submitted). In addition, the nature of TCFs in comparison with the magnetic field has been investigated, and found that the magnetic properties of the materials under the influence of the three distinct TCFs changed significantly (Taheri et al., 2021). Furthermore, the effects of TCFs on in vivo and in vitro models of Alzheimer's disease (Taheri et al., 2021b), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021c), wheat plant (Torabi et al., 2021), bacterial population growth (Taheri et al., 2021d), viral growth (Taheri et al., 2021e), and the electrical activity of the brain during Faradarmani (Taheri et al., 2020b) were studied previously. The aim of this study in addition to the reproducibility of previous results on cell lines was to investigate the effect of Faradarmani CF on 4T1 cell lines under ex vivo conditions to study the effect of the three-dimensional culture conditions on cell behavior in the presence of Faradarmani CF.

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). A request for Connection to the CCN to utilize TCFs can be placed through the COSMOintel website in the "Assign Announcement" section. This access is available for everyone at no cost. In order to study and experience this Connection, the researchers can register on the website at any time and in order to report the experiment to the COSMOintel research center. Certain details of the experiment must be provided to the center; for example, the characteristics or number and name of samples and controls must be specified. This entire experiment was carried out as a double-blind method where lab technicians were completely unaware of TCFs theory, and the Faradarmangar at the COSMOintel research center who established the Connection was unaware of the details of the study. Double-blind is a gold standard that is common in science experiments in the field of medicine and psychology, involving theoretical and practical testing.

2D cell culture

The 4T1 cell line was cultured in a humidified atmosphere with 5% carbon dioxide at 37℃ in Dulbecco modified Eagle medium/Nutrient Mixture F-12 Ham supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin 10,000 U/mL.

Spheroid preparation

The adherent cells were grown to 80% confluence. Monolayers were rinsed twice with PBS. After draining well, 2mL (for 100 mm plates) of 0.05% trypsin-1 mM EDTA was added and incubated at 37℃ until cells detach. Next, the detached cells were transferred to a 15mL conical tube and centrifuged at 1200 rpm for 5 mins. Then, the cell pellet was resuspended in a 2mL complete culture medium, and the cells were counted. Using a pipette, 20 μL of cells were deposited on an inverted lid from a 100mm tissue culture dish. The lid containing 45 deposited cells was inverted in the PBS-filled chamber at 37℃ 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 5min. 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℃ 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℃ 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 after staining with AO/PI (Nexcelom ViaStain™ AO/PI staining solution (Nexcelom, CS2-0106) according to the manufacturer's protocol on day 3.

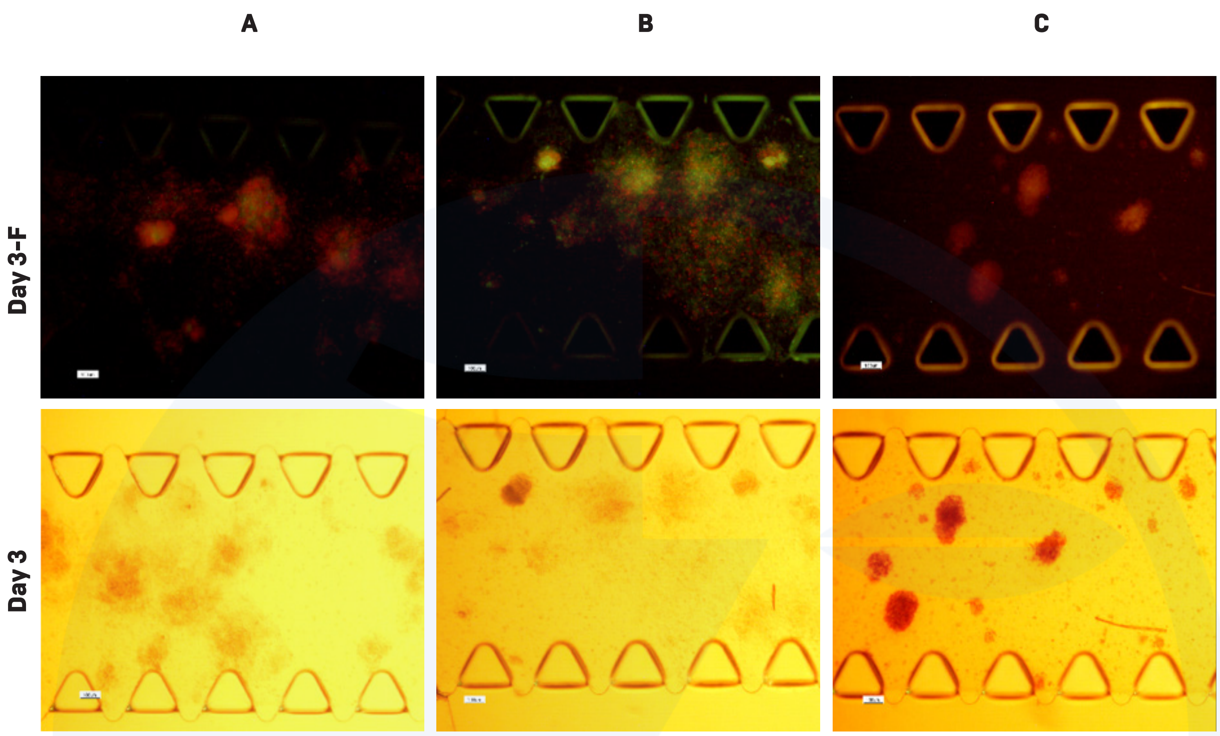
Imaging of spheroids and data analysis

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 Propidum iodide-stained dead (red) cells was quantified.

Results

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)

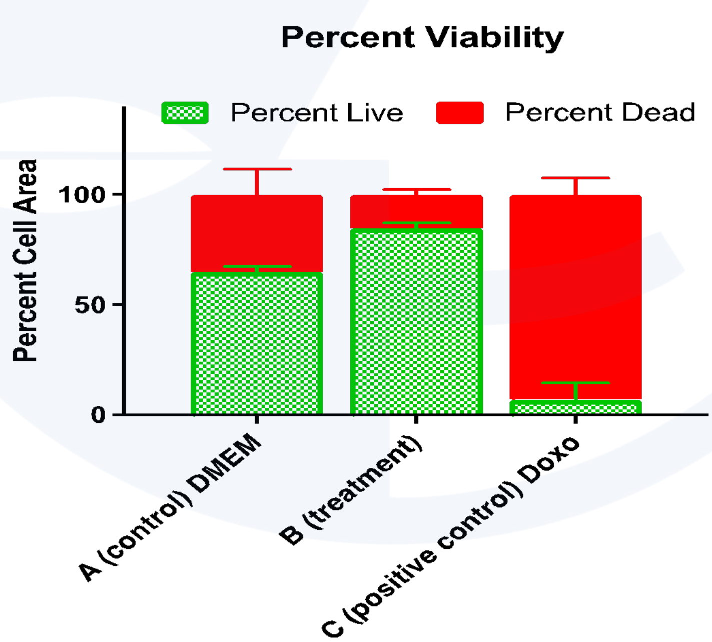


Figure 1. percentage of cell viability on day 3 of the study. A) Optical and fluo escent Images from the spheroids on day 3. Green: live. Red: dead. Scale bars: 100μm. A: DMEM-treated cells (as control), B: Treated cells, C: Doxorubicin-treated cells (as positive control). B)

Discussion and Conclusion

This preliminary study investigated the effect of a Faradarmani Consciousness Field on the growth of a cancer cell line under ex vivo conditions. The results of this study affirmed the findings of the observations made on the in vitro MCF7 culture (Taheri et al., 2020a). The 4T1 cell line of this study shows a significant increase in survival compared to controls. According to Taheri, Faradarmani CF can optimize the system under the study, and this upgrade is due to the function of the target system, and its nature. Due to the independent nature of cancerous cell lines of the cancer cells in the body of living organisms, cancer cell lines have been inclined to their nature and optimized under the influence of Faradarmani CF. Further research is ongoing in this team to examine the primary cancer cells in the same conditions of this study and study the molecular and immunological changes of cells in both cancerous cell lines and primary cancer cells in the face of Faradarmani Consciousness Field.

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