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# Analysis of Cell Cycle in Embryonic Fibroblasts and SW480 (Colon Cancer) under the Influence of Taheri Consciousness Fields

Mohammad Ali Taheri[1] , Sara Torabi[2], Shima Roshani[3], Noushin Nabavi[4] , Farid Semsarha[5*]

1-Sciencefact R&D Department, Cosmointel Inc. Research Center, Ontario, Canada
2-Department of Plant Biology, School of Biology, College of sciences, University of Tehran,Tehran, Iran
3-Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
4-Research Services at University of Victoria, BC, Canada
5-Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran

* Correspondence: Farid Semsarha Ph.D., Institute of Biochemistry and Biophysics (IBB), University of Tehran, P.O. Box: 13145-1384, Tehran, Iran
Tel.: +98-9121786577
Email: Semsarha@alumni.ut.ac.ir

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## Abstract

According to Taheri, applying the Faradarmani Consciousness Field (FCF) can lead to the repair and improvement of any system that is placed under the influence of this T-Consciousness Field. Previously, a growth-inducing effect of the FCF on the MCF7 and 4T1 cancer cell lines was observed under i*n vitro* and *ex vivo* environments respectively. The same cannot be said for *in vivo* experiment as FCF inhibited the growth of tumor in the body of the cancer mouse models. Overall, the results of previous studies confirmed that cancer cell survival and growth is affected by FCF. The present study aimed to evaluate the reproducibility of the observations in previous studies using *in vitro* cell cultures of fibroblast cell line under *Faradarmani Consciousness Field (FCF)* and SW480 cell line under two types of Taheri Consciousness Fields (TCFs). Cell cycle analysis showed that FCF led to a decrease in apoptosis and increase in proliferation of fibroblast cell line. This observation was in accordance with previous studies. Furthermore, according to the MTT assay results, both TCFs 1 and 2 increased survival in the SW480. The flow cytometry data were also consistent with this observation. Cell cycle analysis showed that TCF2 reduced cell survival and the proliferation rate of this cell line. In conclusion, TCFs affected death and survival of these cell lines. Further *in vitro* and *in vivo* studies are necessary to fully understand the precise mechanism of these non-material/non-energetic fields.

**Keywords:** Faradarmani Consciousness Field; Taheri Consciousness Fields; Fibroblast; Cell cycle; Colon cancer, SW480

## Introduction

Embryonic fibroblasts are used for investigating the effects of growth induction factors because of their easy access, handling, and rapid growth rates. Fibroblasts are a group of heterogeneous resident cells of mesenchymal origin that have different locations, diverse appearances, and distinct activities (Qiu et al., 2016). In previous research, according to "Sciencefact" using Taheri Consciousness Fields (TCFs), *in vivo* (Taheri et al., 2022a), *ex vivo* (three-dimensional) (Taheri et al., 2022b) and *in vitro* (two-dimensional) (Taheri et al., 2022c) experiments were conducted. In fact, mainstream science is focused on the physical aspect, or matter and energy. Meanwhile, consciousness—according to Taheri—has a non-physical nature. To distinguish this viewpoint from others, the term T-Consciousness is used here. Therefore, Sciencefact is an approach that, through designing scientific experiments, seeks to reveal the effects of T-Consciousness. Moreover, various T-Consciousness fields with different functions have been introduced, which are subsets of the Cosmic Consciousness Network. Although these fields cannot be directly measured by quantitative instruments, it is possible to record and examine their effects through designing appropriate tests (Taheri et al., 2013). To evaluate the reproducibility of the previously reported results of the influence of TCFs on the cancer cell lines in vitro, we studied the influence of FCF on embryonic fibroblast cells with optimal proliferative capacity using flow cytometry.

Moreover, colorectal cancer is the third most common cancer in the Western hemisphere and its incidence increases with age. Most colorectal cancers with or without lymph node metastasis are local and up to 20% of patients with metastatic disease are more likely to have liver disease (Haraldsdottir et al., 2014). The SW480 cell line was derived from the colon tumor of a 50-year-old Caucasian male patient with colorectal adenocarcinoma. They have an epithelial morphology and high levels of p53, c-myc, K-ras, H-ras, N-ras, sis, myb and fos oncogenes. These cell lines are widely used in biomedical research to aid research and finding a cure for colon cancer (Xiong et al., 2014). In the current study, in addition to fibroblast cell line, the behavior of SW480 under two types of Taheri Consciousness Fields (TCFs) has been investigated.

## Material and Methods

### TCF1 application

In this study, Faradarmani or TCF1 was allocated once every 24 hours for the sample cell culture plates, during the whole study period. Negative control is the fibroblast cells which are untreated with FCF.

### Application of TCFs on SW480 cell line

In this study, the samples treated with TCFs in 12, 24 and 48 hours and this treatment was allocated once every 24 hours for the sample cell culture plates, during the whole study time.

### Cell Culture

The cell lines used for this study were purchased from the Pasteur Institute of Iran. Fetal bovine serum was obtained from Roswell Park Memorial Institute 1640 (Gibco Laboratories, Grand Island, NY) and diluted to 10% using culture media. Penicillin (100 IU/ml) and streptomycin (100 µg/ml) were also supplemented in the culture media (Serox, Germany). Cell cultures were kept in a humid incubator at 37 °C (Memmert, Schwabach, Germany) with 5% $CO_2$. Relative humidity was maintained between 95% and 98% by an atomizer system or water reservoir. Cells were in their logarithmic growth phase for all experiments. The controls in this study were as follows: the negative control consisted of SW480 cells that were not treated with TCFs or the drug, and the positive control consisted of these cell lines treated with doxorubicin.

### MTT Assay

The MTT test was used to evaluate cytotoxicity and cell viability after treatment with TCFs. $3 \times 10^3$ cells were plated in a 96-well culture plate. The effects of TCFs on cell viability were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). For this purpose, MTT (Sigma, Taufkirchen, Germany) at a concentration of 0.2 mg/ml in RPMI-1640 medium was used. The cells were then incubated at 37 °C. After 4 hours, the medium was replaced with 100 µl of dimethyl sulfoxide (DMSO) and 25 µl of Sorenson's buffer (glycine 0.1 M, NaCl 0.1 M, pH 10.5 adjusted with 0.1 M NaOH). The cells were incubated at 37 °C for 30 min, and a microplate reader (Tecan, Sunrise, Switzerland) was used to measure the absorbance at 570 nm.

### Cell Cycle Analysis

Cell cycle progression analysis was performed by staining with propidium iodide (PI). The cells were cultured in 6-well plates ($1 \times 10^5$ cells per well) and kept overnight in a standard incubator. The cells in the experimental group were washed, separated, and harvested, then suspended, fixed in 70% ethanol, and kept at 4 °C for an additional 72 hours. Cells were stained at 37 °C for one hour using 50 µg/ml PI. The proportion of cells at different stages of the cell cycle was assessed using a flow cytometer on the FACSCalibur system (Miltenyi Biotec FACSQuant 10).

### Flow cytometry

Possible changes in apoptosis were measured after treatment with TCFs using the annexin V/propidium iodide (PI) flow cytometry method. A total of $1 \times 10^5$ cells in a 6-well culture plate were used for this assay. After 24 hours, TCF-treated cells were trypsinized and centrifuged at 1,500 rpm for 5 minutes. The cells were then stained with annexin V and PI according to the manufacturer's instructions. For annexin V staining, 2 µl of annexin V, 1 µl of propidium iodide, and 100 µl of binding buffer were added to the samples. The cells were incubated for 15 minutes at room temperature in the dark. The samples were then analyzed by flow cytometry (MACSQuant Analyzer 10, Miltenyi Biotec, Germany). The rate of apoptosis was assessed using FlowJo software (Tree Star, San Carlos, CA).

### Statistical Analysis

Data were analyzed using GraphPad Prism software, version 6.0 (San Diego, CA). All values are presented as mean ± standard error. All analyses were repeated at least three times. To determine the significance of differences, t-tests and analysis of variance (ANOVA) were used, and p-values $< 0.05$ were considered statistically significant.

### Results and Discussion

According to Figure 1, there is a decrease in population of sub G1 stage and an increase in G1 phase in the presence of FCF. No significant changes were seen in the S and G2 phases. In other words, FCF led to a reduction in apoptosis rates and an increase in cell survival in this cell line.

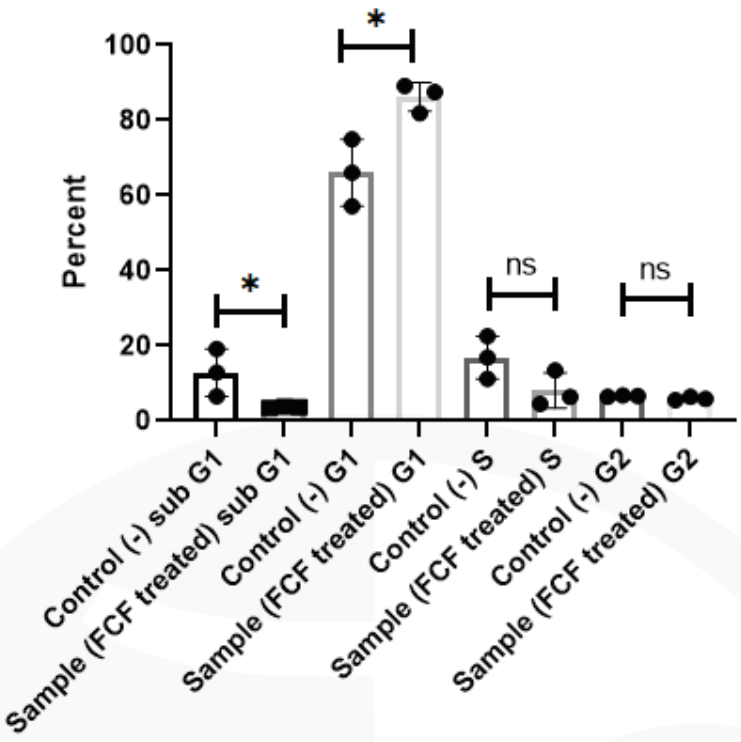


Figure 1. Fibroblast cell cycle analysis under the influence of FCF. *: p-value<0.05, ns: non-significant.

In addition, the MTT assay is used with the aim of measuring cell metabolic activity. The alterations in the behavior of the SW480 cell line at 12, 24 and 48 hours under influence of TCFs compared to the control is presented at Figure 2.

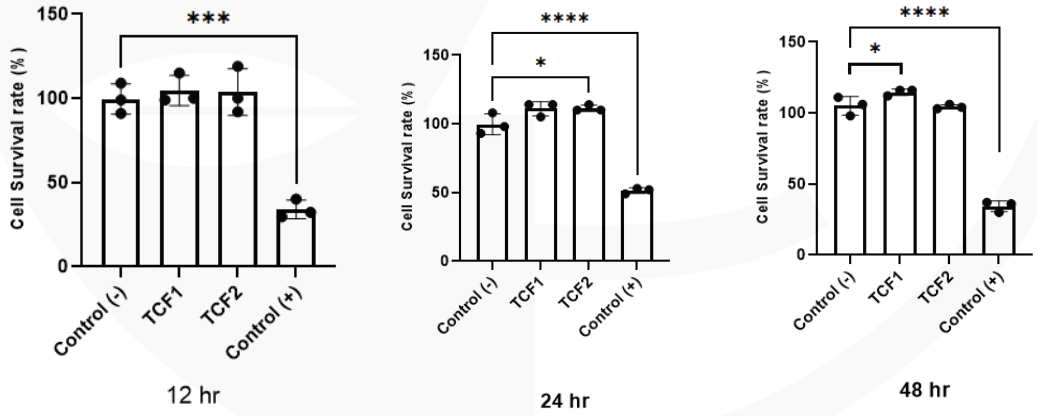


Figure 2. Comparison of the MTT analysis of the SW480 cell line at 12, 24 and 48 hour intervals. (TCF: Taheri Consciousness Field). *: p-value<0.05 ***: p-value<0.001, ****: p-value<0.0001.

As can be seen, SW480 cell line at 24 hours and 48 hours showed increase in survival under the influence of TCF1 and TCF2, respectively. Although the obtained data from MTT assay usually are attributed to the number of viable cells, the rate of tetrazolium reduction represents the metabolic activity of cells such as the rate of glycolytic NADH production (Berridge et al., 2005). So based on the aforementioned results it can be said that there was an increased metabolic activity in SW480 under TCF1 from 12 to 48 hour, and as a result of TCF2 treatment the same behavior was observed at 24 hours. It is to be noted this influence of TCF2 followed

by apoptosis and decreased mitosis at 48 hours. Cell cycle analysis was done at 48 hours. As can be seen in the Table 1, the G2/M phase in the SW480 cell line decreased significantly as a result of TCF2 treatment.

Table 1. Cell cycle analysis of SW480 cancer cell line

| TCF | Cell cycle percentage | | |
|---|---|---|---|
| | G1 | S | G2/M |
| Control (-) | 74.3 | 17.8 | 7.58 |
| TCF1 | 72.3 | 18.8 | 8.17 |
| TCF2 | 89.5 | 8.58 | 1.25* |

*: p-value<0.05

The results obtained from flow cytometry for the SW480 cell line are presented in the Table 2. As observed, the majority of cells, similar to the control sample, were located in the Q4 region. The group exposed to the TCF2 was about 2% lower in the Q4 region, with increases observed in the Q1 and Q3 regions. Considering no significant changes in the regions, the observations of this section are consistent with the MTT assay.

Table 2. Flow cytometry results of cell death in the SW480 cell line.

| % of each cell state | | | | Sample | Cell line |
|---|---|---|---|---|---|
| Q4 | Q3 | Q2 | Q1 | | |
| 98.3 | 0.086 | 0.68 | 0.94 | Control (-) | SW480 |
| 98.1 | 0.18 | 0.37 | 1.35 | TCF1 | |
| 96.7 | 0.92 | 0.9 | 1.77 | TCF2 | |

Here, TCF refers to the T-Consciousness Field; Q1: percentage of necrotic cells; Q2: percentage of late apoptotic cells; Q3: percentage of early apoptotic cells; and Q4: percentage of viable cells.

As it has been explained in the introduction section, the aim of designing experiments in the zero-phase of TCFs research is mainly to report the effects of these novel fields apart from their mechanism at the cellular level. Based on the result, Faradarmani had similar effect on the cell cycle progression of fibroblast cell line and SW40 cell line had different behavior under TCFs compared to the control. These observations warrant more studies, so further investigations about the effect of TCFs on cellular responses will be conducted to test reproducibility.

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