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Effects of Taheri Consciousness Fields on the HT29 Human Colorectal Cancer Cells

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Abstract

Colorectal cancer accounts for 11% of all diagnosed cancers and is the second deadliest cancer worldwide. Surgery, chemotherapy and targeted therapy are generally used for the treatment of colorectal cancer. However, there are significant challenges, such as recurrence of the tumor and drug resistance, so the application of novel methods is required for the treatment of this cancer. T-Consciousness Fields (TCFs) were introduced by Mohammad Ali Taheri. These new fields are neither energy nor matter and cannot be measured directly. However, we can evaluate the effects of TCFs indirectly through various kinds of research in the laboratory. This study aimed to evaluate the effect of TCF-1 and TCF-2 on HT29 human colon cancer cells compared with the control group. The morphology and microscopic properties of cells were investigated in the TCF-1 and TCF-2 groups compared with the control, and the cells were detached and stained with trypan blue, and then the dead cells were counted. To evaluate the effect of the TCFs on cell viability, the MTT assay was used. The expression of two apoptosis-related genes (*Bax* and *Bcl-2*) was assessed using reverse transcription quantitative PCR (RT-qPCR). The results showed that TCFs decreased the number of cells and changed the morphology of HT29 cells. In the trypan blue staining method, more dead cells were counted in the TCF groups compared to the control group. In the MTT assay, both TCFs decreased the viability of cells during incubation times ($p < 0.05$). *Bax/Bcl2* ratio increased 4.9-fold and 7.6-fold in TCF-1 and TCF-2 treated cells, respectively ($p < 0.05$). Therefore, TCF-1 and TCF-2 induced apoptosis in HT29 cells. There are still many ambiguities and questions about the nature and effect of TCFs. To clarify this issue, more research is needed *in vitro, in vivo*, and clinically.

Keywords: Taheri Consciousness Fields, HT29 human colon cancer cells, cell viability

Introduction

The third most common type of cancer is colorectal cancer, and it is the second leading cause of cancer-related mortality rates worldwide. It is estimated that more than 1.8 million colorectal cancer cases and 881,000 related deaths occurred in the world in 2018 (Ferlay et al., 2019). Although the highest incidence of colorectal cancer is still seen in Western economically developed countries, it has recently increased rapidly in other parts of the world (Brenner et al., 2018). Although modern research has elucidated the pathogenesis of colorectal cancer and provided advanced screening strategies, the incidence of colorectal cancer continues to increase (Siegel et al., 2020). The current standard treatment of colorectal cancer is removing the tumor with surgery, then adjuvant chemotherapy or targeted therapy. However, tumor recurrence and drug resistance are important challenges in treating colorectal cancer (Wolpin et al., 2008; Gao et al., 2021). The median overall survival of patients with metastatic colorectal cancer is approximately 30 months, indicating a poor prognosis in these patients (Van Cutsem et al., 2016). For this reason, innovative and more effective approaches are essential for the treatment of metastatic disease.

According to Taheri's theory, T-Consciousness is the main component of the universe, and its matter, energy, and information are derived from it. According to this theory, there are various TCFs with different functions that have a non-frequency nature. These fields cannot be directly measured by science, but their effects can be investigated across various subjects through reproducible laboratory experiments. The effect of TCFs begins with the brief and immediate attention of the Faradarmangar (trained individual) to the subject of study (Taheri, 2013).

In previous research, we observed the effects of the TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021a), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021b), and the electrical activity of the brain during Faradarmani in the Faradarmangars population (Taheri et al., 2020b).

This study aimed to evaluate the effect of T-Consciousness Fields (1 and 2) on HT29 human colon cancer cells compared with the untreated control group. The comparison was made by counting and evaluating the number of dead cells, morphology, and microscopic characteristics in cell culture. MTT test to compare cell viability in the treated and untreated groups was performed. Also, quantitative evaluation of *Bax* apoptotic and *Bcl-2* anti-apoptotic genes expression using reverse transcription quantitative PCR (RT-qPCR) was performed.

Methods and Materials

Application of the Taheri Consciousness Fields

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.COSMOintel.com). More details are provided in the Common Considerations section of this issue. Announcement programs in this study are shown in Table 1.

Table 1. Announcement programs for the TCFs treatment and control groups.

Groups	Number of Announcements	Announcement intervals after seeding of cells		
TCF-1	2	20 min	24 hours	
TCF-2	3	20 min	24 hours	48 hours
Control	0	-	-	-

Cell culture and study of the morphology and microscopic properties of cells

The HT29 human colorectal cancer cells are not only utilized to investigate the biology of human colorectal cancers but also receive special interest

in studies of food bioavailability and digestion because of their capability to express properties of mature intestinal cells (Martínez-Maqueda et al., 2015). HT29 human colorectal cancer cells were obtained from the National Cell Bank of Iran. RPMI 1640 media (Gibco, Germany) with 10% fetal bovine serum (FBS) (Gibco, Germany) and 1% streptomycin/ penicillin (Sigma Aldrich, Germany) was utilized for cell culture, and the cells were incubated at 37 °C, 90% humidity, and 5% CO2. The morphology and microscopic properties of cells were evaluated in the TCFs treatment and control groups.

Trypan blue staining assay

Trypan blue is a vital stain that stains dead cells blue, leaving living cells unstained, as seen under a microscope. Living cells do not absorb stains because they have a healthy cell membrane (Ludford, 1929).

For the trypan blue staining assay, cells were detached from 6-well plates by trypsin, then an equal volume of cell suspension and trypan blue (0.4%) was mixed and incubated for three minutes at room temperature. Then, dead cells were counted using a hemocytometer, and the ratio of living to dead cells was calculated. Three replications were considered for all examinations. The formula for the calculation of the percentage (%) of dead cells stained with trypan blue (Strober, 2015):

$$\text{Dead cells (\%)} = (A_{\text{Dead cells}}/A_{\text{All counted cells}}) \times 100$$

MTT assay

MTT assay was used to determine the effect of the TCF-1 and TCF-2 on the viability of HT29 cells. First, the HT29 cells were seeded in 96-well microplates (10,000 cells per well). After 24 hours for the TCF-1 group and 48 hours for the TCF-2 group, the medium in the wells was replaced with MTT solution (0.5 mg/mL), and the microplates were incubated at 37 °C under 5% CO2 for 4 hours. Then, the MTT solution was replaced with 100 µL dimethyl sulfoxide (DMSO). A plate reader (BDSL Immunoskan MS, Finland) was used to measure the absorbance of the wells at 570 nm. All tests were repeated three times. To evaluate the cell viability, the following formula was utilized (Chueh et al., 2014):

$$\text{Cell Viability (\%)} = \frac{OD\ test - OD\ blank}{OD\ control - OD\ blank} \times 100$$

Evaluation of gene expression by the RT-qPCR method

The expression of *Bax* and *Bcl-2* in HT29 cells was assessed by RT-qPCR using the Favor Prep total RNA Isolation Kit (Favorgen, Taiwan). Extraction steps were performed according to the kit instructions. After extracting RNA from each sample, the quantity and purity of the obtained RNA were evaluated through the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).

The cDNA was synthesized using a cDNA synthesis kit (Biotechrabbit GmbH, Germany) following the manufacturer's instructions. The qPCR reaction was performed in a MIC real-time PCR system (BioMolecular Systems, London, UK). The final volume of each reaction was 20 µl, containing 10 picomoles of reciprocating primers for the *Bax* and *Bcl-2* genes, 40 ng cDNA, and 10 µL SYBR green PCR master mix (Ampliqon, Denmark). In this study, the *GAPDH* gene was considered the reference gene. The primer sequences used in the present study are listed in Table 2.

Table 2. Name and properties of primers used in qPCR.

Target Gene	Sequence of primers	Length	Annealing Temperature
Bax	F: 5'-TTGCTTCAGGGTTTCATCCAG-3' R: 5'-AGCTTCTTGGTGGACGCATC-3'	101 bp	65
Bcl-2	F: 5'-TGTGGATGACTGAGTACCTGAACC-3' R: 5'-CAGGCAGGAGAAATCAAACAGAG-3'	122 bp	66
GAPDH	F: 5'-CGTCTGCCCTATCAACTTTCG-3' R: 5'-CGTTTCTCAGGCTCCCTCT-3'	74 bp	63

For the PCR reaction, the following conditions were used: the first step was incubation at 94°C for 12 min, followed by 40 cycles for amplification, each cycle consists of a denaturation step for 15 seconds at 94°C, an annealing step for 15 seconds at 62-67°C, and an extension step for 10 seconds at 72°C. Melting curve analysis was performed to confirm the specificity of amplicons. The comparative gene expression was calculated with the standard $2^{-\Delta\Delta ct}$ (Livak et al., 2001; Arocho et al., 2006).

Statistical analysis

In this study, all tests were repeated three times and calculated as mean ± SD. One-way ANOVA was applied to evaluate the TCFs effects on cell viability, gene expression level examinations, and $p < 0.05$ was considered the significant level.

Results

Evaluation of morphology and microscopic properties of cells

In the microscopic examination of HT29 cells in the TCFs groups, the cell colonies were smaller, and the cells were rounder, and less accumulation was seen. Also, the time required for cells to detach after trypsinization from the plate, which is usually three to five minutes, was reduced to two minutes in the treatment group (this could be due to cell weakness and mortality). The cell confluency of the TCFs groups was significantly lower than that of the control group, and after incubation times, there were still empty spaces in the cell culture plate. Figure 1 shows microscopic evaluations of control HT29 cells and cells exposed to the TCF-2 at 24 and 48 hours after culture.

Trypan blue staining assay

The number of dead cancer cells of both TCFs groups in trypan blue dye was counted on average at least 10% more than the control group.

MTT test results of HT29 human colon cancer cells

MTT assay was used to evaluate the viability of TCF-1- and TCF-2 treated cells. Results showed that both TCFs significantly decreased cell viability compared with the control group (Figure 2) ($p < 0.05$).

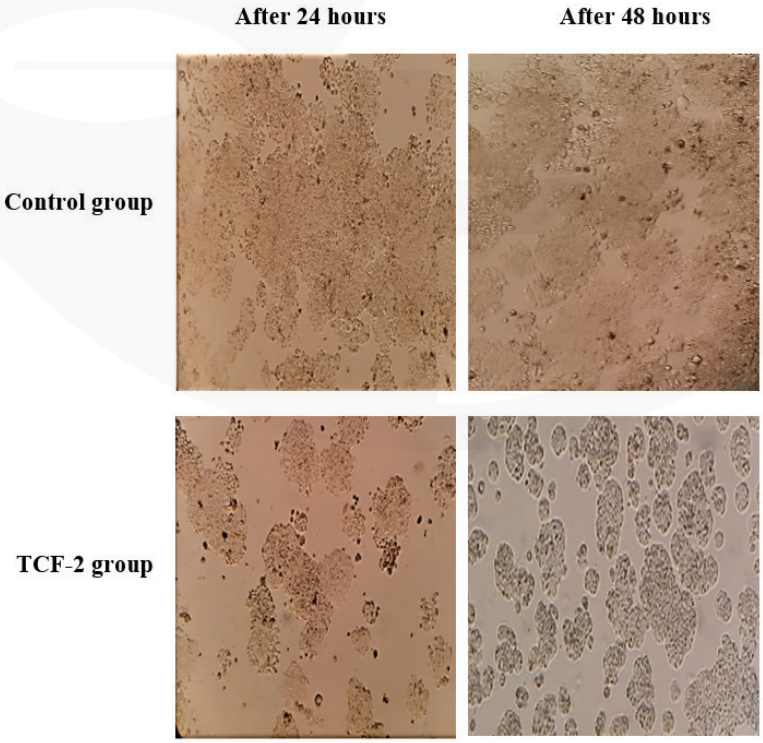


Figure 1. Microscopic evaluation of control HT29 cells and cells exposed to the TCF-2 at 24 and 48 hours after culture. There is more intercellular space in the TCF-2 group compared to the control group.

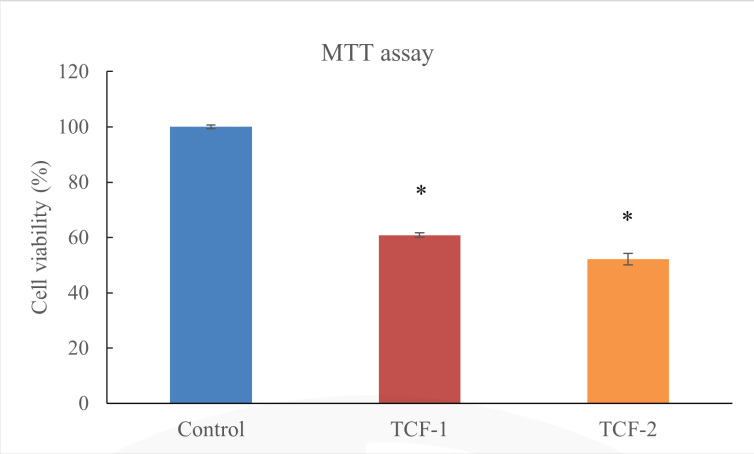
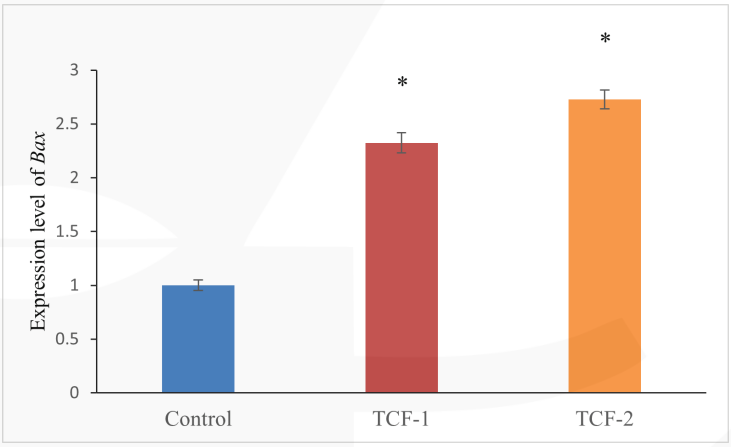
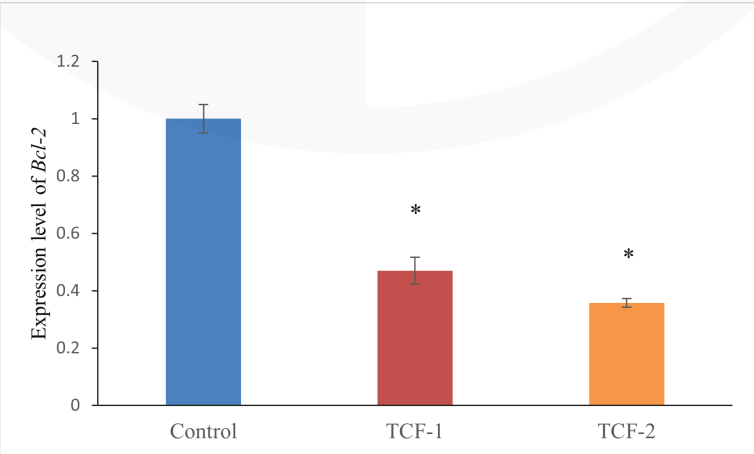


Figure 2. Cell viability of TCF-1 and TCF-2 treated groups compared with the control. The asterisk shows a statistically significant difference ($p < 0.05$) between the experimental and control groups.

Expression of *Bax* and *Bcl-2* genes in TCF-1 and TCF-2 treated HT29 cells

This study showed the change in the expression of *Bcl-2* and *Bax* genes and the ratio of *Bax/Bcl- 2* in TCF-1 and TCF-2 treated HT29 cells. The expression of the *Bax* gene was increased in both TCF-1, and TCF-2 treated cells compared with control (Figure 3a). The *Bcl-2* expression was significantly decreased in both TCFs groups ($p < 0.05$; Figure 3b). *Bax/Bcl-2* ratio was significantly increased in the TCFs treated cells ($p < 0.05$; Figure 3c).





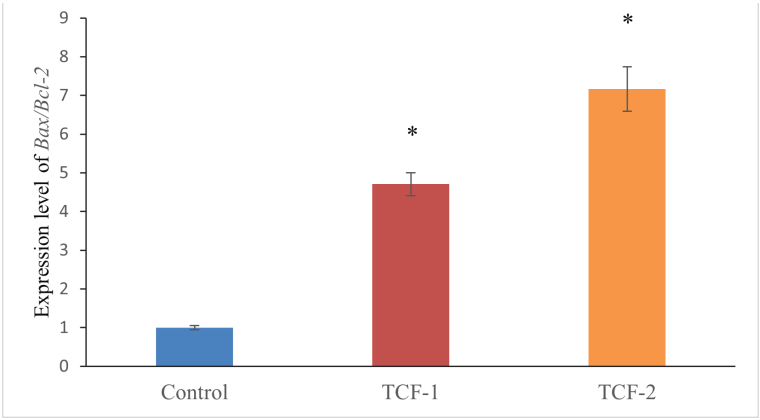


Figure 3. The expression of *Bax* (a) and *Bcl-2* (b) and the ratio of *Bax/Bcl-2* (c) in TCF-1 and TCF-2 treated cells compared with control. The asterisk displays a significant difference between the TCFs groups and the control ($p < 0.05$).

Discussion

In the present study, TCF-1 and TCF-2 considerably influenced the number of cells and the morphology of HT29 human colon cancer cells. In the trypan blue staining assay, more dead cells were counted in the TCFs groups compared with the control. This may indicate an inducing effect of the TCFs on HT29 cell death. In the MTT assay, both TCFs decreased cell viability at the specified incubation times ($p < 0.05$).

Bcl-2 family members play a critical role in regulating cell death or survival. Chemotherapeutic agents partially affect the expression of numerous members of the Bcl-2 family in cancerous cells. Members of the Bcl-2 family regulate apoptosis, with Bax acting as a pro-apoptotic factor and Bcl-2 acting as an anti-apoptotic factor.(Green et al., 1998; Rao et al., 1997).

The expression of *Bcl-2* and *Bax* genes and the *Bax/Bcl-2* ratio were evaluated in the present study. The results showed that *Bax* gene expression increased and *Bcl2* gene expression decreased in both TCF-1 and TCF-2 groups compared to the control. The ratio of *Bax/Bcl-2* is an indicator of cell susceptibility to apoptosis and helps determine cell fate (Gross, 2001). The *Bax/Bcl2* ratio increased 4.9-fold and 7.6-fold compared to control via TCF-1 and TCF-2, respectively ($p < 0.05$), indicating that apoptosis induced by both TCFs was significant.

The effect of Faradarmani CF on MCF7 cells was assessed in a previous study. The Faradarmani CF was announced every hour until the end of the experimentation at 6, 18, and 24 hours from the initial start time. Cell viability was assessed using the MTT assay. The expression of the *Bax* and *Bcl-2* genes was assessed using RT-qPCR in MCF-7 breast cancer cells. Faradarmani CF significantly increased MCF-7 cell proliferation (18%) compared to the control in a time-dependent manner. Furthermore, in cells treated with Faradarmani CF, the *Bax/Bcl2* ratio decreased (1-fold) compared with the control, suggesting higher MCF7 cell survival and resistance to death (Taheri et al., 2020a).

The influence of TCFs on cancer cells seemingly displays different results. Both of the TCFs showed a significant anticancer effect. It is necessary to further study TCFs and perform laboratory tests in a variety of areas. Therefore, extensive studies *in vitro* and *in vivo* are needed to evaluate the efficacy of TCFs on cancer, and further studies are recommended.

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Conflicts of Interest

The authors declare no conflict of interest.

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