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T-Consciousness Fields Inhibit Apoptosis and Promote ATP Production in HEK-293 Cells Under Microgravity Stress

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Abstract

T-Consciousness Fields (TCFs) are introduced as non-physical entities, with their effects initiated through the human mind. It is hypothesized that information transmitted via TCFs may influence the behavior of studied subjects. The present experiment aimed to investigate the effects of TCFs on cell cycle progression and ATP production in the HEK-293 cell line under both microgravity (MG) and Earth gravity (1G) conditions. To achieve this, cultured cells were exposed to TCFs for 24 hours following an initial 24-hour incubation period, under either microgravity (MG) conditions, simulated by clinostat rotation, or a standard Earth gravity (1G) environment. Untreated cells served as the control group. Flow cytometry was used to assess the distribution of cells across different phases of the cell cycle, and ATP concentration was measured by evaluating luciferase enzyme activity. The results showed a significant increase in the sub-G1 phase under microgravity (MG) conditions ($p < 0.05$), indicating elevated apoptosis. In contrast, TCFs-treated samples maintained sub-G1 levels comparable to those under Earth gravity (1G). Additionally, TCFs treatment significantly increased the S phase under MG ($p < 0.05$) and the G2 phase under 1G ($p < 0.05$). ATP concentrations were markedly reduced under MG in both treated and untreated samples compared to 1G ($p < 0.05$). However, in the clinostat environment, ATP levels in TCFs-treated samples were approximately twice as high as those in untreated controls ($p < 0.05$), whereas no significant difference was observed under 1G conditions. These findings suggest that the reduced sub-G1 phase in TCFs-treated samples under MG reflects apoptosis inhibition, while increased ATP levels and prolonged S phase indicate enhanced cellular efficiency under stress. The distinct outcomes under MG and 1G imply that the information transmitted by TCFs may vary depending on environmental conditions. Further studies are needed to elucidate the underlying mechanisms of TCF action.

Keywords: T-Consciousness Field, Microgravity, Clinostat, Cell cycle, Information

Introduction

It is well-documented that reduced gravity can have adverse effects on human physiology (Mochi et al., 2022). Indeed, space exploration has presented scientists with numerous challenges, many of which stem from microgravity-induced alterations in cellular responses. Consequently, researchers have long sought therapeutic interventions to safeguard the health of astronauts during extended space missions (Nguyen et al., 2021). One promising approach to understanding the impact of microgravity on biological systems is the investigation of cellular parameters such as proliferation, apoptosis, and cell cycle progression (Sokolovskaya et al., 2014).

The cell cycle, a fundamental process in eukaryotic cells, has been the focus of over a century of research. It consists of four main phases: G1, S, G2, and M (Wang, 2022). Disruptions in cell cycle regulation, as well as in programmed cell death mechanisms, can lead to irreversible health consequences and are implicated in the development of various disorders, including cancer, inflammation, neurodegenerative diseases, and cardiovascular conditions (Wiman and Zhivotovsky, 2017).

Life on Earth has evolved under constant gravitational force, and the human body is naturally adapted to this terrestrial environment (Adamopoulos et al., 2021). As a result, space travel introduces a range of physiological threats. For instance, alterations in the extracellular microenvironment of cancer cells under microgravity conditions can stimulate the secretion of cytokines and tumor growth factors, thereby potentially increasing cancer malignancy (Kim et al., 2021). Moreover, significant changes in the expression of genes related to oxidative stress, inflammation, and cell cycle regulation have been observed in mice during the STS-131 space shuttle mission, which may contribute to cardiac dysfunction (Kumar et al., 2021).

According to Taheri, T-Consciousness is a fundamental element of the universe. Within this framework, various T-Consciousness Fields (TCFs) exist, each serving distinct functions as subcategories of the Cosmic Consciousness Network. These fields cannot be measured using conventional quantitative instruments; however, their effects can be observed and recorded through laboratory experiments conducted on a range of subjects. The influence of TCFs is initiated through the human mind, requiring only a brief moment of attention, typically just a few seconds (Taheri, 2013).

These fields can be applied to both living organisms and inanimate materials. In our previous studies involving animal, plant, and cell models, we observed that the properties and behaviors of TCF-treated samples were noticeably altered compared to untreated controls. It is hypothesized that these observed changes result from the transmission of information from the TCFs (Taheri et al., 2023; Torabi et al., 2023; Taheri et al., 2024). The aim of this study was to investigate the effects of three types of T-Consciousness Fields (TCFs) on cell cycle progression and ATP production in the HEK-293 cell line under both microgravity (MG) and normal Earth gravity (1G) conditions.

Material and methods

T-Consciousness Fields application

Three types of TCFs, including TCF1, 2 and 3, were applied to the samples according to protocols regulated by the COSMOintel research center (www.COSMOintel.com). Further details are provided in general consideration section of this issue.

Microgravity (MG) application

In this study, microgravity (MG) conditions were simulated using a clinostat, which was donated by the United Nations Office for Outer Space Affairs in Vienna to the Aerospace Research Institute of Iran. Although the gravity vector cannot be eliminated on Earth's surface,

MG (approximately 10^{-6} g) can be effectively simulated using this device. The clinostat was first sterilized with ultraviolet light and 70% ethanol, then placed inside an incubator set to 37 °C. After an initial 24-hour incubation period, cultured cells were treated with T-Consciousness Fields (TCFs) under either MG or normal Earth gravity (1G) conditions. Four experimental groups were established (n = 3 per group): MG with TCFs (Group 1), MG without TCFs as control (Group 2), 1G with TCFs (Group 3), and 1G without TCFs (Group 4). The samples without TCFs were considered as control. The TCFs treatment was initiated simultaneously with clinostat rotation and continued for 24 hours.

Cell Culture

In this study, the HEK-293 cell line—characterized by epithelial morphology and originally derived from the kidney of a human embryo—was obtained from the Pasteur Institute of Iran. Cells were cultured in a 6-well plate (4×10^5 cells per well) using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Incubation was performed at 37 °C in a humidified atmosphere containing 5% CO_2.

After 24 hour, the medium in each well was removed, and the wells were washed with phosphate-buffered saline (PBS). A 1X solution of 0.25% trypsin-EDTA was then added to each well. The plate was incubated at 37 °C for 5 minutes to allow cell detachment. Trypsin activity was neutralized by adding 300 µl of complete culture medium. The cells were then collected, centrifuged at 1200 rpm for 5 minutes, and the resulting cell pellet was stored at –80 °C until ATP assay analysis. ATP concentration was measured using a luminometer (Berthold Technologies GmbH & Co. KG) based on luciferase enzyme activity.

In this study the following materials were used: ATP (Roche), D-luciferin potassium salt (Resem, The Netherlands), Fetal bovine serum (FBS) (BIO-IDEA), Dulbecco's modified Eagle's medium (DMEM) (BIO-IDEA), Penicillin/streptomycin (BIO-IDEA), Trypsin-EDTA 25% (BIO-IDEA), Tris-HCl (Merck), NaOH (Merck), MgSO4 (Merck), PMSF

Cell Lysis

To lyse the cells, 30 µl of CCLR buffer (containing 50 mM Tris, 150 mM NaCl, 1% Triton X-100, and 0.1 mM PMSF; pH 6.9) was added to the cell pellets. The samples were incubated on ice for approximately 20 minutes, followed by centrifugation at 13,000 rpm for 15 minutes at 4 °C. The resulting supernatant was then collected and used for the ATP assay.

ATP assay

An ATP standard curve was first established by preparing a serial dilution of ATP within the concentration range of 0.001–1 mM. To eliminate any ATP contamination, the luciferase enzyme was dialyzed in 50 mM Tris buffer for 24 hours. For ATP quantification in the treated cells, equal volumes (1:1:1 ratio) of the dialyzed luciferase enzyme, luciferin, and cell lysate were mixed in a tube. The resulting luminescence was measured using a luminometer (Jouaville et al., 1999).

Flow Cytometry

Harvested cells were washed twice with PBS and resuspended in 50 µl of cold PBS (2–8 °C) using brief vortexing. The cells were then fixed in 1 ml of cold 70% ethanol (–20 °C) and thoroughly mixed with a vortex mixer. Following fixation, the cell suspension was centrifuged at 1500 rpm for 20 minutes at room temperature. After discarding the supernatant, the cells were washed once with PBS. Subsequently, PBS was carefully removed, and 1 ml of MIX MASTER PI solution was added, ensuring a final cell concentration of 5×10^5 cells/ml. The cells were then incubated at room temperature for 30 minutes before analysis. The distribution of cells across different stages of the cell cycle was assessed using a BD FACSCalibur flow

cytometer (BD Biosciences, San Jose, CA, USA). Data analysis was performed with FlowJo software (Tree Star, San Carlos, CA, USA).

Master PI mix solution for cell cycle

Propidium iodide (PI) 1 mg/ml: 40 µl; RNase (DNaseFREE) 10mg/mL: 10 µl; PBS, ca+2, mg+2 Free: 950 µl.

Statistical Analysis

Each experiment was conducted in triplicate, and the data are presented as mean ± standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by multiple comparisons with a 95% confidence interval, using GraphPad Prism software (version 8). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

As shown in Table 1, the highest percentage of live cells based on the FSC channel was observed in the TCF-treated samples under microgravity (MG) conditions, indicating a resistance to MG-induced stress. Additionally, Figure 1 (a) shows that elevated SSC-H values in the untreated microgravity (MG) sample may indicate the induction of structural damage or apoptosis. Assessing changes in the Sub-G1 phase of the cell cycle provides insight into whether cells are healthy or undergoing apoptosis. In the clinostat environment, the proportion of cells in the Sub-G1 phase was approximately twice that of the control under 1G conditions.

Environmental stress factors in space such as MG can pose a threat to the DNA integrity of living organisms (Moreno-Villanueva et al., 2017) and induce apoptotic cell death (Singh et al., 2021; Kossmehl et al., 2003). Apoptotic cells are usually associated with morphological changes and reduced DNA content, which can be detected by flow cytometry (sub-G1 DNA content) (Plesca et al., 2008). In such conditions, DNA repair pathways are activated, including cell cycle arrest, which provides time for DNA repair (Zhou and Elledge, 2000; Prasad et al., 2020). In the current study, an increase in the sub-G1was not observed in the TCF-treated samples. These findings suggest that TCF treatment may mitigate the adverse effects of MG stress on cell cycle progression.

Table 1. Effects of T-Consciousness Fields (TCFs) on the distribution of cell cycle phases under microgravity (MG) and Earth gravity (1G) conditions.

Sample	Live cells based on FSC channel (%)	Sub G1	G1	S	G2	Super G2
MG-Control	61.85±2.83	13.41±0.86	41.14±1.15	28.44±2.47	23.17±0.02	0.61±0.45
MG-TCFs	74.70±2.87	7.49±0.21	38.61±0.60	33.70±1.65	21.89±0.27	0.42±0.08
1G-Control	67.70±1.56	8.96±1.94	43.28±3.71	29.94±0.45	19.89±2.63	0.80±0.04
1G-TCFs	67.40±2.25	8.68±0.40	39.19±1.36	29.43±0.05	25.63±0.76	0.56±0.34

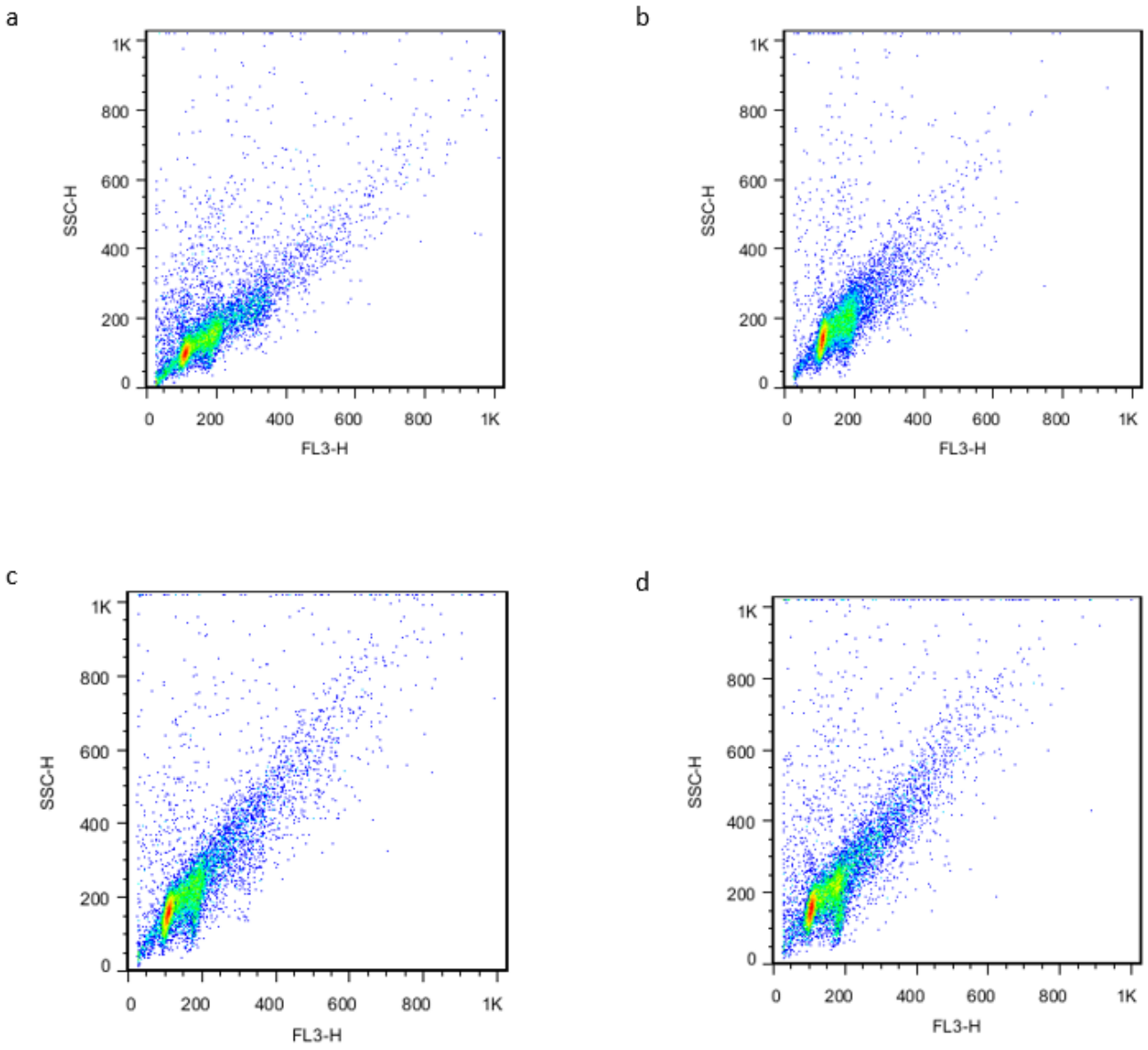

Figure 1. Representative histograms showing flow cytometry analysis using the FL3-A channel. (a) Microgravity (MG) without TCFs treatment, (b) MG with TCFs treatment, (c) 1G without TCFs treatment, (d) 1G with TCFs treatment.

Statistically significant changes are shown in Figure 2. TCFs treatment resulted in a downward trend in the G1 phase and a significant increase in the S phase. Previous studies have shown that MG stress can induce G1 phase arrest, leading to impaired cell cycle progression and reduced cellular proliferation (Quynh Chi et al., 2020; Hoang et al., 2025). In the present study, the observed increase in the S phase, accompanied by a decline in the G1 phase, suggests that TCFs treatment facilitates the transition from G1 to S phase under MG conditions. This indicates a potential protective or adaptive role of TCFs in promoting cell cycle progression and mitigating MG-induced cell cycle arrest.

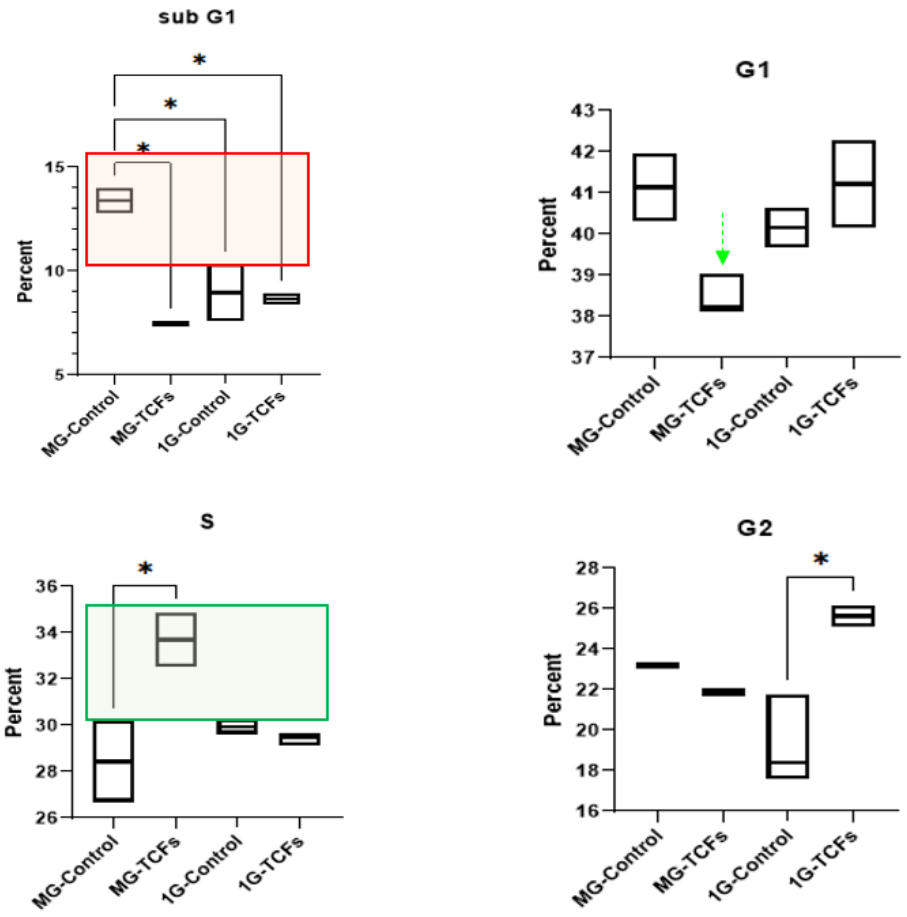

Figure 2. Box plot showing the effect of T-Consciousness Fields (TCFs) on the percentage distribution of cell cycle phases. Green and red boxes indicate changes in trend relative to the 1G control. *: p-value < 0.05. MG: microgravity

The behavior of cells under the influence of TCFs in normal Earth gravity differed from that observed under microgravity conditions. Notably, the longest G2 phase was found in TCFs-treated cells under 1G. The G2 phase is the final part of interphase, during which cells prepare for mitotic division by duplicating organelles, such as mitochondria, and synthesizing proteins essential for cell division (Mascanzoni et al., 2019). Given that no significant changes were observed in the S phase or in overall cell proliferation in TCFs-treated cells under 1G, the prolonged G2 phase may reflect a form of cell cycle arrest. This finding warrants further investigation into organelle dynamics and other cellular changes under TCFs treatment to better understand the underlying mechanisms.

The concentrations of ATP and luciferase activity in the experimental groups are presented in Table 2 and Figure 3. MG stress significantly reduced ATP production in both the control and TCFs-treated samples, with an approximate 96% decrease in the control group compared to the 1G control. However, under MG conditions, TCFs treatment markedly increased ATP levels—by up to 115%—compared to the untreated MG control. In contrast, TCFs treatment under Earth gravity (1G) did not lead to a significant change in ATP concentration.

Table 2. Changes in luciferase activity and ATP concentration with and without T-Consciousness Fields (TCFs), compared to their levels under Earth gravity (1G).

Sample	Control/1G	TCFs+/1G	Control/MG	TCFs+/MG
Luciferase activity Mean/ RLU.s^{-1}	9,966,667	9,833,333	3,383,333	5,540,000
Std. Deviation	1,322,876	927,362	440,076	733,485
[ATP]Mean /µM	9.472	9.194	0.379	0.816
Std. Deviation	1.608	2.009	0.049	0.185
$\frac{\text{([ATP] Control 1G)}}{\text{([ATP] Sample)}}$	1	1.03	24.99	11.61

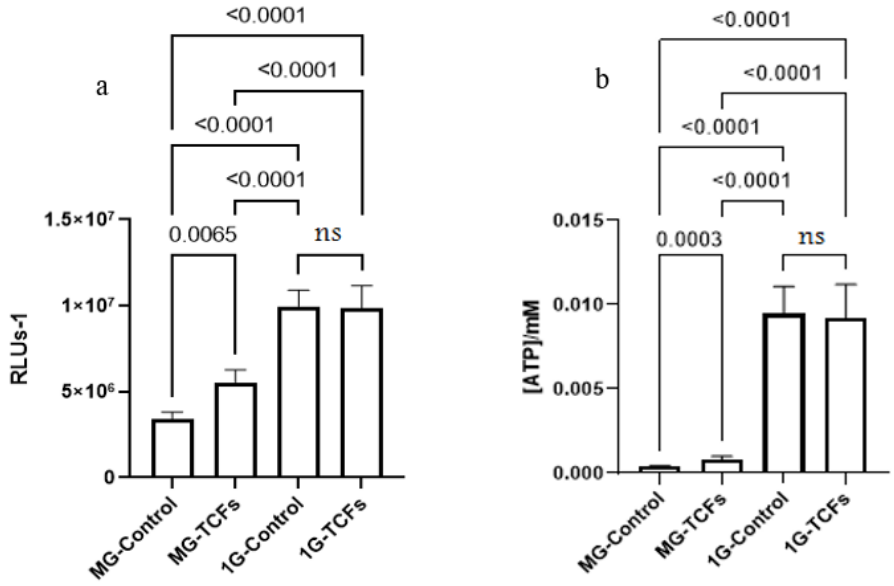

Figure 3. Effects of T-Consciousness Fields (TCFs) on a) the activity of luciferase enzyme and b) the concentration of ATP under microgravity (MG) and Earth gravity (1G) conditions. Vertical bars indicate mean ± standard error of three replicates. P-value < 0.05 considered as significant, and ns shows non-significant change.

Microgravity stress markedly decreased ATP concentrations compared to the 1G condition. As shown in Table 2, ATP levels under MG were approximately 25-fold and 12-fold lower in the control and TCF-treated cells, respectively. Despite this significant reduction, the percentage of viable cells was not significantly affected. Notably, although ATP concentration in TCF-treated cells under MG decreased by about 91% relative to the 1G control, cell viability was approximately 6% higher than under normal gravity. We hypothesize that, in the absence of gravity, cells may exhibit improved metabolic efficiency or altered energy utilization mechanisms.

This observation is consistent with several studies suggesting that, in the absence of gravity, cells may adapt by optimizing energy utilization and potentially requiring less ATP to maintain essential functions. For example, plants grown under microgravity conditions have shown altered gene expression related to cell wall synthesis, oxidative stress responses, and energy

metabolism—adaptations that may help sustain growth and development (Soleimani et al., 2019; Baba et al., 2022). Similarly, certain bacterial species exhibit gene expression changes that enhance their resilience to microgravity stress, potentially contributing to more efficient energy utilization (Milojevic et al., 2020).

Conclusion

In conclusion, this study showed that TCFs differentially affect cell cycle progression and ATP production in HEK-293 cells under simulated microgravity (via clinostat) and Earth gravity conditions. While microgravity stress induced an increase in the sub-G1 phase—indicating apoptosis—TCFs-treated cells exhibited a pattern more similar to those in normal gravity. Furthermore, TCFs treatment led to significantly higher ATP levels under microgravity, suggesting enhanced energy utilization or protection against MG-induced metabolic disruption. What if TCFs can prevent the damaging effects of microgravity on human cells? TCFs may offer a novel, non-invasive strategy to support astronaut health in space. Further studies in real microgravity and even hypergravity environments are needed to explore this potential and determine whether TCFs could become a valuable tool in future space exploration.

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