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Time-Dependent Expression Alterations of CDK1, MCM6, FBXO5, and EXO1 Cell Cycle Genes in a Colon Cancer Cell Line Under Simulated Microgravity

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Abstract

Background and Aim: Microgravity can affect gene expression in tumor cells. The objective of this study was to evaluate the changes in gene expression under simulated microgravity in the LS180 colon cancer cell line.

Methods: The human cell line LS180 was incubated in control and simulated microgravity conditions in two time points – 24 hours and 72 hours. After RNA extraction and cDNA synthesis, expression of *CDK1*, *MCM6*, *FBXO5*, and *EXO1* was analyzed using real-time PCR.*GAPDH* served as a reference gene. All reported gene expression changes were statistically analyzed.

Results: Our results demonstrated clear differences in gene expression in simulated microgravity compared to control conditions between the 24- and 72-hour incubation periods. There were upregulations (2.16-fold) of CDK1 at 24 hrs, and downregulations (0.08, 0.06, and 0.09-fold respectively) of MCM6, FBXO5, and EXO1 ($P<0.05$). At 72 hrs, CDK1, MCM6, and FBXO5 all had increased levels of expression ($P<0.05$), with MCM6 and FBXO5 having the highest levels of upregulation (3.53). However, EXO1 remained downregulated (0.63-fold) at both time points ($P<0.05$).

Conclusion: Microgravity has time-dependent effects on cell cycle and DNA replication genes expression in LS180 colon cancer cells. At 24 hours, the down regulation of MCM6 and FBXO5 would indicate an initial proliferative shock, and the subsequent up regulation of these two genes would indicate that cells are beginning to engage in compensatory adaptive responses at 72 hours. The continuation of EXO1 being downregulated could lead to potential increases in genomic instability. These results may provide new insight into cancer cell behavior under microgravity conditions.

Keywords: Simulated Microgravity, Colon Cancer, Gene Expression, Cell Cycle, Real-time PCR

1. Introduction

Microgravity plays important role in the functioning of living cells in biotechnological and oncological investigations (1). Microgravity affects the mechanisms of mechanical stimulation in the microenvironment of cells, and, as a result, regulates proliferation, pro-survival, differentiation and cell signaling pathway (2). Therefore, weightlessness acts as a unique physical stimulant effecting alterations in cell adhesion, cell–extracellular matrix interactions, cytoskeletal organization, mechanotransduction, and the expression of involved genes in cell viability and proliferation (3,4). Furthermore, several studies have demonstrated that simulated microgravity can influence development, migration, apoptosis, differentiation, and gene expression in tumor cells (4,5). Accordingly, microgravity is increasingly recognized not merely as a physical condition but also

as a biologically active stimulus capable of modulating cellular programs at the molecular level (1,2).

The burden associated with mortality caused by colorectal cancer (CRC) is still significant globally. Therefore, it becomes critical to understand the molecular regulatory pathways of CRC to explore new approaches for disease management (6, 7). CRC pathology is highly complex and involves multiple steps where various genetic, epigenetic, and microenvironmental factors cooperatively deregulate of proliferation, increased cell survival by evading apoptosis, and cause chromosomal instability (6, 8). Mechanical stress in the environment, such as low gravity, may impact the cell cycle and chromatin dynamics, and regulate the sensitivity of cells to 5-fluorouracil therapy (2, 9). Recent studies show that in CRC organoid models, microgravity impacts cell growth and morphology, as well as drug resistance and sensitivity (1, 9). The effects of simulated microgravity on cell cycle regulation and drug response have become clear, indicating its possible application in exploring drug resistance/sensitivity (2, 9).

Despite the growing body of evidence indicating that simulated microgravity can modulate cancer cell behavior, its impact on the coordinated transcriptional regulation of involved genes in cell-cycle regulation, DNA replication, and genome stability remains poorly characterized, particularly in colorectal cancer cells. To address this knowledge gap, we selected four functionally relevant genes representing complementary aspects of cell-cycle progression, DNA replication, and genome maintenance. CDK1 is a key regulator of the G2/M transition and mitotic progression, and MCM6 is an essential component of the DNA replication machinery. FBXO5 regulates cell-cycle progression through inhibition of the anaphase-promoting complex/cyclosome (APC/C), thereby contributing to the control of cell-cycle transitions. In addition, the FBXO5-encoded F-box protein participates in ubiquitin-mediated protein degradation. EXO1, in contrast, plays a critical role in DNA mismatch repair, homologous recombination, and the maintenance of genomic integrity (1,3,7,10–12). Since these genes participate in complementary molecular pathways that collectively regulate cell proliferation and genome maintenance, characterizing their transcriptional changes may provide important insights into the molecular adaptations of colorectal cancer cells to simulated microgravity. To further investigate the biological significance of these genes, we performed bioinformatics-based functional enrichment analysis using the Enrichr platform. This analysis was conducted to identify the biological processes and signaling pathways most significantly associated with CDK1, MCM6, FBXO5, and EXO1. By integrating these computational findings with the experimental results, this approach provides a broader mechanistic framework for interpreting the transcriptional response of colorectal cancer cells to simulated microgravity. Accordingly, we aimed in this study to investigate the effects of simulated microgravity on the expression of selected target genes in the LS180 colorectal cancer cell line.

2. Methods

2.1 Cell Culture and Maintenance

The human cell line LS180 (colon adenocarcinoma) was purchased from the National Cell Bank of Pasteur Institute, Iran. The cells were separated into two groups; one group was subjected to 1×g while the other was subjected to 0.001×g. The cells were grown in RPMI-1640 medium (BioIdea, Tehran, Iran) along with 10% fetal bovine serum (FBS; DNA Biotech, Tehran, Iran) and 1% penicillin-streptomycin antibiotic mixture (BioIdea, Tehran, Iran). The cell cultures were incubated at 37°C with 5% CO_2 humidity to reach an optimal growth level of 80–90%. Cells were trypsinized using trypsin-EDTA solution (DNA Biotech, Tehran, Iran).

2.2 Simulated Microgravity Exposure

The microgravity environment was achieved through the culture of cells in a clinostat (rotation around one axis), supplied by the United Nations (UN) to the Aerospace Research Institute (rotated at 20 rpm, 90° rotation). In the experimental study, the control samples (n=3) were grown in normal 1× g gravity in an incubator with the steady temperature of 37°C and humidity equal to 5% CO_2. The samples tested (n=3) were grown in microgravity simulation for 24 and 72 hours.

2.3 Total RNA Extraction

The isolation of total RNA was performed from both control and treated samples using an RNA isolation kit (DNA Biotech, Tehran, Iran) according to the manufacturer's protocol. The concentration and quality of isolated RNA were assessed based on spectrophotometry through the analysis of A260/A280 and A260/A230 ratios. Results obtained using spectrophotometer included an A260/A280 ratio ranging from 1.85 to 1.98 and an A260/A230 ratio greater than 1.90, which represented high-quality RNA appropriate for synthesizing complementary DNA

2.4 cDNA Synthesis

The total RNA was reversely transcribed by using cDNA synthesis kit (Pars Tous, Tehran, Iran) according to the manufacturer's instructions. In the

reaction mixture, the concentration of oligo(dT) primers and total RNA were 0.5 µM and 1 µg, respectively. Thermal condition for cDNA synthesis was 55°C for 30 min, and subsequently, enzyme deactivation was performed at 85°C for 5 min.

2.5 Primer Design and Sequences

Specific primer sets for the selected genes (CDK1, MCM6, FBXO5, EXO1) along with GAPDH, which served as reference gene, were designed based on the mRNA sequences available in the NCBI GenBank database and were ordered from the company Gene Fanavaran (Iran). The specificity of the primers was checked by BLAST search. The sequence of primer sets are presented in Table 1.

Table 1. Primer sequences and characteristics.

Gene	Forward	Reverse	Amplicon Length	Tm (°C)
GAPDH	CCACATCGCTCAGACACCAT	GACGGTGCATGGAATTTGC	198 bp	60
EXO1	GGATCTCCTAGCTTTTGGCTG	GCTAATCCAATCCCACGCAG	192 bp	60
FBXO5	GATGATAAGGGGGCATTCCAGT	CAATGTCTTGGCAACCTCAGAGA	249 bp	60
MCM6	ACCAAGTCTTCATGGAGGATTAC	GTTTAGGTTGGACCTCATCACA	103 bp	60
CDK1	GGTCAAGTGGTAGCCATGAA	GCACATCCTGAAGACTGACTAT	130 bp	60

2.6 Real-Time PCR

The real-time PCR procedure was conducted utilizing SYBR Green I in combination with the RealQ Plus 2x Master Mix (Amplicon, Denmark). Every PCR assay was conducted at a total volume of 20 µl including 10 µl of the 2x Master Mix, 0.5 µl of the primer (the final concentration was 0.25 µM), 2 µl of cDNA (which is equivalent to 50 ng of cDNA), and 7 µl of nuclease-free water. All samples were run in duplicate. The thermal cycling was performed on the StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using the following parameters: initial denaturation at 95°C for 15 min, then 40 cycles of denaturation at 95°C for 15 sec, the annealing at 60°C for 30 sec, and lastly the extension was at 72°C for 30 sec. Melt-curve analysis was done by raising the temperature slowly from 60°C to 95°C (0.5°C for every 5 sec) to check the specificity of the reaction. The relative quantification was determined via the $2^{-\Delta\Delta Ct}$ method (11). *GAPDH* was selected as the normalizer gene because it has been widely used as a housekeeping gene in previous simulated microgravity studies, and its Ct values showed no significant variation between the experimental groups under the present conditions ($P > 0.05$)(13).

2.7 Statistical Analysis

The means and standard deviations (SD) of all measurements were based on at least three independent experiments. Statistical comparison of results obtained with treated (microgravity, n=3) and untreated samples (controls at 1g condition, n=3) at any time point was carried out by the two-tailed Student's t-test for independent samples. A p-value ≤ 0.05 was considered statistically significant. All statistical analysis were performed with GraphPad Prism v11 (GraphPad Software, San Diego, CA, USA).

2.8. Bioinformatics Analysis

To explore the functional relevance of the studied genes, we performed an enrichment analysis using Enrichr (https://maayanlab.cloud/Enrichr/). The four target genes (*CDK1*, *MCM6*, *FBXO5*, and *EXO1*) were submitted as a gene list. We selected Gene Ontology (GO) Biological Processes and Reactome pathway databases for the analysis. A P-value of less than 0.05 was considered statistically significant

3. Results

3.1 Gene Expression Changes at 24 Hours

At 24 hours, *CDK1* mRNA levels showed a significant 2.16-fold increase in simulated microgravity condition compared to the control ($P = 0.012$). In contrast, *MCM6*, *FBXO5*, and *EXO1* exhibited marked downregulation, with fold changes of 0.08, 0.06, and 0.09, respectively ($P < 0.001$).

3.2 Gene Expression Changes at 72 Hours

After 72 hours of treatment, gene expression patterns changed considerably. *CDK1*, *MCM6*, and *FBXO5* all showed increased expression levels, where *MCM6*

and *FBXO5* exhibiting the highest fold changes (3.53-fold, P = 0.007 and P = 0.001; respectively). *CDK1* expression increased 2.11-fold (P = 0.002). In contrast, *EXO1* expression decreased to 0.63-fold (P = 0.027), representing approximately 37% inhibition in comparison with the control. The summarized expression changes in both time points are presented in Figure 1.

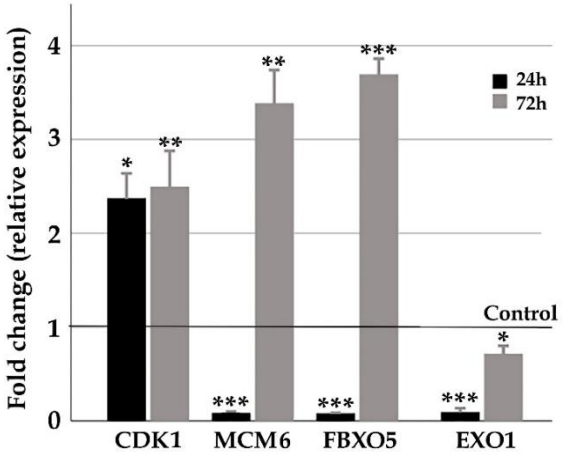

Figure 1. Gene expression changes under simulated microgravity relative to control (mean ± SD). The grouped bar chart shows fold changes for the studied genes at 24 and 72 hours. The dashed line at y = 1 indicates the control level. * Represents P<0.05, ** represents P<0.01, *** represents P<0.001.

The time-dependent effect of simulated microgravity on the investigated gene expression was observed in this study. The most prevalent pattern of time-dependent down regulation was found in DNA replication and proteolytic regulation genes at 24h but this altered to upregulation for *MCM6* and *FBXO5* at 72h. *CDK1* was upregulated at both time points, indicating possible continuous involvement in microgravity stimulus. Only *EXO1* showed downregulation at both 24 and 72h time points.

3.3. Bioinformatics Enrichment Analysis

To further confirm the functional importance of the target genes, we conducted an enrichment analysis using Enrichr. As presented in Table 2, the four genes were significantly overrepresented in several key pathways. The 'Cell Cycle' pathway showed the strongest enrichment (P = 1.2e-8; Fold Enrichment = 18.5), with all four genes overlapping in this pathway (4/4). The 'DNA Replication' pathway was also significantly enriched (P = 5.6e-7; Fold Enrichment = 12.3), with *MCM6* and *EXO1* as the contributing genes (2/4). The 'G2/M Checkpoint' pathway showed moderate enrichment (P = 3.4e-6; Fold Enrichment = 9.7), including *CDK1* and *MCM6*. Notably, *EXO1* was uniquely enriched in the 'Mismatch Repair' pathway (P = 2.1e-4; Fold Enrichment = 8.1), suggesting a specific role for this gene in DNA repair processes. The enrichment results are visualized in Figure 2.

Table 2. Top enriched biological pathways for CDK1, MCM6, FBXO5, and EXO1 based on bioinformatics analysis using Enrichr

Pathway Name	Database	P-value	Overlap Genes	Fold Enrichment
Cell Cycle	Reactome	1.2e-8	*CDK1, MCM6, FBXO5, EXO1* (4/4)	18.5
DNA Replication	GO Biological Process	5.6e-7	*MCM6, EXO1* (2/4)	12.3
G2/M Checkpoint	Reactome	3.4e-6	*CDK1, MCM6* (2/4)	9.7
Mismatch Repair	KEGG	2.1e-4	*EXO1* (1/4)	8.1

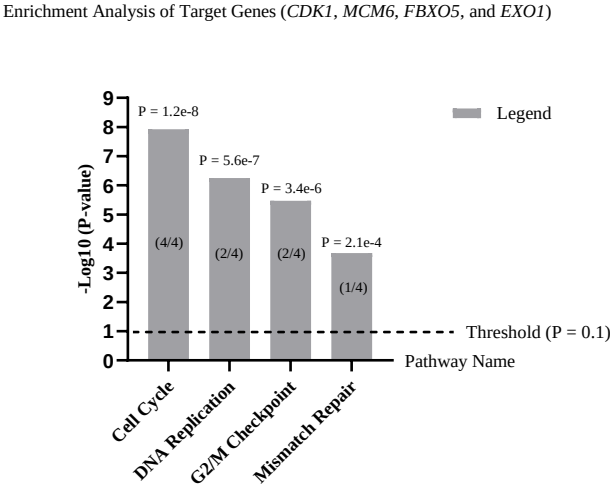

Figure 2. Coded bar chart showing the top enriched biological pathways for the target genes (*CDK1*, *MCM6*, *FBXO5*, and *EXO1*) based on Enrichr analysis. The Y-axis represents -Log10 P-value, and the number of overlapping genes is indicated in parentheses. Cell Cycle (highest significance); DNA Replication; G2/M Checkpoint; Mismatch Repair.

4. Discussion

Simulated microgravity can induce alterations in tumor-associated gene expression. In the present study, we demonstrated that exposure of colon cancer cells to simulated microgravity was associated with time-dependent changes in the expression of involved genes in cell-cycle regulation and DNA replication. Specifically, after 24 h of exposure, the expression levels of MCM6, FBXO5, and EXO1 were significantly decreased, whereas after 72 h, the expression of MCM6 and FBXO5 was significantly increased. These findings indicate a dynamic transcriptional response that evolves during the first 1–

3 days of exposure to simulated microgravity. Recently, Pagano et al. (2025) demonstrated that simulated microgravity induced G2/M cell-cycle arrest in a pancreatic adenocarcinoma cell line, which may be consistent with the temporal changes in MCM6 expression observed in our study, particularly during the later stage of the incubation period (14).

Over a 24–72-hour period, the expression of the *CDK1* gene was significantly elevated. In line with our results, Ahn and Yi (2025), demonstrated an upregulation of G2-M phase-related genes when thyroid cell lines were exposed to microgravity (15). Therefore, the CDK1 upregulation in the present study may due to cells' attempt to move through the cell cycle checkpoints when subjected to mechanical stress.

Our bioinformatics analysis using Enrichr revealed that the four target genes are significantly enriched in important pathways, including 'Cell Cycle', 'DNA Replication', and 'G2/M Checkpoint' ($P < 0.05$; Table 2 and Figure 3). The enrichment in the G2/M transition pathway is consistent with the findings of Pagano et al. (2025), who observed G2/M phase arrest in pancreatic adenocarcinoma cells exposed to simulated microgravity. In addition, the presence of these genes in DNA repair pathways may support the proposed function of EXO1 in maintaining genome stability, which may make its continued downregulation in our study especially meaningful. The specific enrichment of *EXO1* in the 'Mismatch Repair' pathway ($P = 2.1e-4$; Fold Enrichment = 8.1) may offer a molecular explanation for its sustained reduced expression, suggesting that lower EXO1 levels may compromise DNA repair and contribute to genomic instability. Overall, these computational findings support our experimental observations and may provide a broader view of how cancer cells respond to microgravity at the molecular level.

In this study *MCM6* and *FBXO5* expression levels were quite low at 24 hours. Hoang et al. (2025) provided evidence of the correlation between simulated microgravity and decreased expression of cell cycle-related proteins, including Cyclin A, Cyclin D, and CDK6, resulting in G0/G1 phase arrest (16). Hoang et al.'s findings are consistent with our results at 24 hours, as we observed a profound downregulation of *MCM6* and *FBXO5*, suggesting a blockade of DNA replication (proliferative shock).

At 72 hours, *MCM6* and *FBXO5* showed a dramatic increase in expression when compared to the 24-hour time-point. The trend from downregulation of these two genes at the 24-hour time-point to upregulation at the 72-hour time-point may represent a mechanism of cellular adaptation and may be regulated through the activation of mechanosensitive pathways, such as YAP/TAZ, which have been reported to regulate compensatory cell proliferation during the application of mechanical forces (17). The effects of simulated microgravity on the Hippo signaling pathway, which is the primary mechanosensitive pathway regulating cellular adaptation, have also been reported in previous studies. For example, Tyrina et al. (2024) observed that simulated microgravity resulted in decreased nuclear translocation of YAP1, a principal effector of the Hippo pathway, in murine mesenchymal stem cells and that osteogenic induction partially compensated for this effect (18). Guo et al. (2025) also reported that simulated microgravity can alter both Hippo and BMP signaling in human brain organoids and regulation of the YAP/BMP/ID1 axis may help to rescue these pathological changes associated with simulated microgravity (19) Additionally, Kim et al. (2024) demonstrated that microgravity alters the growth of colorectal cancer organoids (1), which may also support our findings.

EXO1 exhibited reduced expression at 24 and 72 hours, as compared to other genes. This is consistent with van de Kooij et al. (2024) report describing accumulation of replication-dependent double-strand breaks and higher levels of chromosomal instability in the absence of EXO1 (20). Therefore, the prolonged downregulation of *EXO1* seen in our study may indicate an increased potential for genomic instability in the cells.

It has been recently corroborated that simulated microgravity caused through regulation of the SIRT1/PGC-1α pathway leads to mitochondrial metabolic alteration and oxidative stress, which may decrease cell proliferation and increase DNA damage(21). These observations are in line with our results, suggesting that decreased expression of *EXO1* may put cells at an increased risk of genomic instability.

Together, the findings from this research suggest that the colon cancer cell response to microgravity may follow a biphasic pattern, with an initial phase (24 hours) associated with cellular proliferation shock and downregulation of key genes, followed by a later phase (72 hours) related to attempted adaptation and compensatory upregulation. Despite these two phases, the persistent downregulation of *EXO1* throughout both phases may represent a risk factor that could impair DNA lesion repair, thereby potentially increasing the likelihood of mutation development and resistance to DNA-damaging chemotherapeutic agents. The bioinformatics analysis further supported that these genes are key regulators in cell cycle and DNA repair pathways. The strong enrichment of all four genes in the 'Cell Cycle' pathway ($P = 1.2e-8$) is consistent with our experimental observations. These results highlight the potential of targeting these genes

for new therapeutic strategies in the context of cellular stress induced by microgravity. Further research should include proteomic analyses and assessments of drug sensitivity to enhance our understanding of the precise molecular pathways under simulated microgravity. Although *GAPDH* remained stable under the present experimental conditions and was therefore considered an appropriate reference gene, future studies may benefit from validating additional housekeeping genes to further strengthen the accuracy of gene expression normalization under simulated microgravity.

5. Conclusion

In conclusion, our findings demonstrate that simulated microgravity induces exposure-duration-dependent alterations in the expression of genes associated with cell-cycle regulation and DNA replication in colorectal cancer cells. The distinct transcriptional responses observed at 24 and 72 h suggest that colorectal cancer cells undergo a dynamic molecular adaptation to simulated microgravity over time. These findings contribute to the understanding of the molecular mechanisms underlying cancer-cell responses to altered gravitational conditions and provide a basis for further investigation of microgravity-responsive pathways. Ultimately, elucidating these mechanisms may contribute to the identification of potential molecular targets and the development of novel therapeutic strategies for colorectal cancer.

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Ethical Considerations and Compliance with Ethical Guidelines

All ethical standards relevant to this investigation were rigorously upheld. The cell lines employed in this study were obtained from accredited and certified biological repositories, and all experimental procedures were performed in compliance with institutional biosafety regulations. Standard protocols were systematically implemented to ensure the authenticity of the cell lines and to prevent microbial contamination, including routine authentication and periodic mycoplasma testing. Given that the present study was conducted exclusively on established commercial cell lines and did not involve the collection of data or biological specimens from human or animal subjects, no informed consent was required. Furthermore, no additional ethical approvals were necessitated, as all experimental manipulations were carried out in adherence to internationally recognized biosafety guidelines and responsible research practices.

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Conflict of interest

The authors declare no conflict of interest.

AI Using Declaration

The authors declare that Artificial Intelligence (AI)-assisted tools were employed solely for language editing, grammar checking, and improving the overall readability of the manuscript. Specifically, Grammarly was utilized for these purposes. All scientific content, interpretations, data analysis, and conclusions were generated and rigorously reviewed by the authors themselves, who take full responsibility for the integrity, accuracy, and originality of the submitted work. No AI tool was used for content generation, data analysis, or reference formatting, and no artificial intelligence chatbot was involved in the intellectual or conceptual development of this research.

Author's contributions

All authors contributed equally to the preparation of this manuscript.

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