Mini Review

Effects of Simulated Microgravity on Anti-Cancer Drug Responsiveness

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Abstract: This review examines the effects of simulated microgravity on cancer cells and their response to anticancer drugs. In the unique environment of space, characterized by near-weightlessness, biological systems function differently compared to Earth's normal gravitational conditions, potentially altering drug efficacy. As human space exploration advances, understanding pharmaceutical behavior in microgravity becomes essential for astronaut healthcare. We present comprehensive findings on how microgravity conditions, simulated using technologies such as the Rotary Cell Culture System and 3D clinostats, affect cancer cell behavior and drug sensitivity. The review analyzes how microgravity influences anticancer drug effectiveness, with evidence suggesting increased drug sensitivity in certain cancer types through mechanisms involving membrane property alterations, drug transport modifications, and signaling pathway changes. We discuss key experimental findings across various cancer models, including leukemia, gastric, ovarian, and colorectal cancers, while addressing methodological limitations of microgravity simulation research. This synthesis of current knowledge advances our understanding of cancer treatment in space environments and may offer novel insights for terrestrial therapeutic strategies.

Keywords: microgravity; 3D clinostat; anticancer agents

1. Introduction

In the extreme environment of space, biological phenomena differ from those under normal gravitational conditions on Earth, which may consequently alter the efficacy of pharmaceuticals. As we prepare for an era of manned space exploration, it is essential to acquire foundational knowledge in biomedical sciences that can support the health of astronauts and space tourists. There is a need to study the pharmacological properties of various drugs, including anticancer agents, under conditions that simulate the space environment. Microgravity, one of the extreme conditions in space, characterized by a state of near-weightlessness, presents a distinct physical environment that can profoundly influence biological systems.

The investigation of microgravity's effects on cancer cells has been facilitated by the development of simulated microgravity platforms, enabling researchers to conduct experiments under controlled laboratory conditions. These platforms, such as the Rotary Cell Culture System (RCCS) and 3D clinostats, mimic the reduced gravitational forces experienced in space, allowing for the observation of cellular responses in a controlled setting. Studies utilizing these platforms have revealed that microgravity can induce a range of cellular effects, including morphological alterations, changes in gene and protein expression, and modulation of metabolic and signaling pathways.

This review aims to provide a comprehensive overview of current research on the effects of simulated microgravity on the response to anti-cancer drugs. Initially, we will explore the tools and techniques employed to simulate microgravity environments and conduct cellular studies. We will then discuss the impact of microgravity on cancer cells and summarize current reports on its effects on anticancer drug responsiveness. By synthesizing existing knowledge in this field, this paper seeks to enhance our understanding of cancer treatment in space environments.

2. Experimental Methods for Simulating Microgravity

The study of microgravity's effects on biological systems has been made possible through the development of sophisticated simulation platforms. These methods are often classified into four categories: orbiting microgravity facilities, non-orbiting microgravity facilities, ground microgravity simulators, and ground microgravity analogs [1]. Among these, the RCCS and the 3D clinostat—both belonging to the ground microgravity simulators category—were selected as the primary focus of this review.

The RCCS, originally developed by NASA, represents a significant advancement in microgravity simulation technology. This system operates by vertically rotating a High Aspect Ratio Vessel (HARV) around a horizontal axis, generating solid-body rotation of both the vessel and the cell culture medium. This rotation effectively randomizes the gravity vector, creating a simulated microgravity environment. The RCCS is particularly valuable for its ability to provide a low-shear cell culture system, minimizing mechanical stress on cells during experimentation [2–4].

The 3D clinostat, also known as a random positioning machine (RPM), offers an alternative approach to microgravity simulation. This device features two independently rotating frames that operate along perpendicular axes, providing continuous rotation that creates time-averaged simulated microgravity. The system functions by continuously changing the direction of gravity, effectively mimicking the reduced gravitational forces experienced in space [5,6].

3. Effects of Microgravity on Cancer Cells

The influence of microgravity on cancer cells manifests through multiple mechanisms, affecting various aspects of cellular behavior and function. Research has revealed complex patterns of response that vary depending on cancer cell type and experimental conditions. Understanding these effects is crucial for both space pharmacy and potential therapeutic applications.

Cell growth and proliferation responses to microgravity demonstrate variability across different cancer types. For example, conflicting results have been reported in the literature. Studies have shown that microgravity can significantly inhibit the proliferation of lymphoma cells, with time-averaged simulated microgravity (taSMG) resulting in marked reductions in cell division rates [7]. Similarly, melanoma cells exposed to microgravity conditions show decreased proliferation rates compared to those maintained under normal gravity [8]. However, this response is not universal across all cancer types. Colorectal cancer cell organoids, for instance, exhibit enhanced proliferation under simulated microgravity conditions [5], suggesting that the effects of microgravity on cell proliferation are highly dependent on cancer cell type and specific cellular characteristics. Recent review papers provide comprehensive summaries of microgravity effects on cancer cells [9].

The impact of microgravity on cellular morphology and structure represents another significant area of investigation. One of the most notable effects is the formation of three-dimensional spheroids when cells are cultured under microgravity conditions [10–13]. Various cancer cell lines with adherent characteristics form multicellular spheroids when exposed to microgravity [14]. As intuitively expected, when cells cannot adhere to the culture vessel bottom due to weightlessness, they assemble with each other, resulting in spheroid formation. These spheroid structures often demonstrate increased size through both cell division and fusion processes, accompanied by enhanced expression of cell-adhesion molecules. The cytoskeleton undergoes substantial reorganization in response to microgravity, with microtubules losing their preferential orientation toward the cell periphery. These structural changes can have profound implications for cell mobility and drug response. These effects are also summarized in the review paper mentioned above. [9].

4. Microgravity-Induced Alterations in Drug Response

The interaction between microgravity and cancer drug response represents a complex and fascinating area of study with significant implications for both space pharmacy and terrestrial cancer treatment. Research has revealed that microgravity conditions can substantially modify how cancer cells respond to various therapeutic agents, potentially offering new approaches for enhancing treatment efficacy.

Drug sensitivity under microgravity conditions exhibits significant differences compared to normal gravity environments. The mechanisms underlying these changes in drug sensitivity seem to involve alterations in cellular membrane properties, drug transport systems, and intracellular signaling pathways. The reported effects of microgravity on anticancer drug sensitivity are summarized in Table 1, while Table 2 presents associated cellular changes, including modifications in migration patterns and cytoskeletal organization under drug treatment conditions.

Among studies examining anticancer drug responses under microgravity conditions, some research has been conducted in actual space environments. The Lewis group investigated the effects of microgravity on multidrug

resistance using HL60 and HL60/AR (anthracycline-resistant) leukemia cell lines during the Space Shuttle flight STS-67. Their study revealed that microgravity significantly altered cellular responses to doxorubicin treatment. Flight cells showed disrupted cytoskeletal organization with reduced tubulin polymerization, resulting in an amorphic globular shape. Notably, both HL60 and HL60/AR flight cells contained significantly higher amounts of doxorubicin than their ground controls, suggesting impaired drug efflux mechanisms under microgravity conditions. While HL60/AR cells maintained some ability to eliminate doxorubicin even in microgravity, the overall findings indicated that weightlessness affects drug resistance mechanisms, possibly through cytoskeletal reorganization and altered membrane dynamics [15].

Ekpenyong's group investigated how microgravity affects cancer cell responses to chemotherapy using HL60 and K562 human leukemia cell lines [2]. They cultured cells under both normal gravity (1G) and simulated microgravity conditions using HARVs in an RCCS. Cells were exposed for 48 h, then treated with clinically relevant concentrations of two major anthracyclines: daunorubicin and doxorubicin. Both drugs significantly enhanced cell migration while maintaining cell viability above 95% and showing no significant changes in cell size. The researchers chose a 6-h migration period to match physiologically relevant timeframes for cellular extravasation and tissue migration. The findings demonstrate that microgravity can modify cancer cell responses to chemotherapy, particularly in terms of cell migration behavior.

In another study by Ekpenyong's group [3], they conducted a comprehensive investigation into how simulated microgravity affects drug response in K562 cancer cells using the RCCS. The study focused on two therapeutic agents, hydroxyurea and paclitaxel, revealing distinct patterns of cellular response under microgravity conditions. A significant finding was that microgravity inhibited the typical reduction in nuclear-to-cytoplasmic (N/C) ratio normally observed with hydroxyurea treatment, while paclitaxel treatment showed no significant changes in N/C ratio under the same conditions. This differential response suggests that microgravity's influence on drug efficacy may be drug-specific. The N/C ratio, serving as a crucial indicator of cell morphology, reflects important changes in cell cycle, growth, and differentiation patterns.

Kulbacka's group investigated the effects of a simulated microgravity environment on the cisplatin resistance of ovarian cancer cells using a 3D clinostat. The experimental results showed that under simulated microgravity conditions, the migratory and invasive abilities of SKOV-3 ovarian cancer cells were diminished. Cytoskeletal rearrangement was noted alongside changes in cell morphology, such as a rounded shape, membrane blebbing, the presence of lamellipodia, and a lack of filopodia. Exposure to simulated microgravity induced G1/G0 cell cycle arrest in SKOV-3 cells, although this did not significantly affect cell viability. Clonogenic assays demonstrated that the combination of simulated microgravity and cisplatin treatment reduced cell proliferation. This study suggests that microgravity can influence cellular pathways related to the cell membrane and cytoskeleton, potentially enhancing the chemosensitivity of ovarian cancer cells. [6]

In a separate study, the same group investigated simulated microgravity's effects on gastric cancer cells using the RCCS. The research focused on two specific gastric cancer cell lines: EPG85-257 RDB, known for its drug resistance, and EPG85-257 P, which is drug-sensitive. The RCCS was operated at a rotational speed of 20 rpm to simulate a microgravity environment, and the cells were treated with doxorubicin. The study found that under microgravity conditions, there was a notable decrease in the expression of genes associated with drug resistance. Additionally, an increase in DNA/RNA damage was observed, indicating that microgravity may influence genetic stability. The researchers also confirmed a reorganization of the cellular cytoskeleton, which is significant as it suggests structural changes within the cells. Furthermore, there was an increase in the cells' sensitivity to chemotherapy under microgravity conditions, along with an overall reduction in cell viability. These findings suggest that microgravity can potentially weaken drug resistance in gastric cancer cells and enhance the effectiveness of chemotherapy [4].

Recent studies have reported on the effects of microgravity on tumor organoid cultures. Ku's lab conducted research using four organoid models derived from colorectal cancer cells to study drug responses and transcriptomics. By employing a 3D clinostat to simulate microgravity conditions, researchers discovered that CRC organoids generally exhibited increased cell viability and proliferation under microgravity compared to normal gravity controls. The organoids were exposed to simulated microgravity for 24 h, followed by treatment with nine anticancer drugs—5-FU, afatinib, AZD2014, buparlisib, irinotecan, olaparib, MK5108, oxaliplatin, and SAHA—for 72 h. Transcriptomic analysis revealed significant changes in the TBC1D3 gene family and cell cycle-related pathways. Notably, drug screening showed enhanced sensitivity to 5-FU under microgravity conditions, while responses to other drugs varied among the different organoid lines [5].

Table 1. Effects of simulated microgravity on the anti-cancer drug response.

Simulated Microgravity	Cell Line	Cell Type	Drug	Findings	Reference
RCCS	EPG85-257 RDB, EPG85-257 P	Gastric cancer	Doxorubicin	Increased susceptibility	[4]
3D Clinostat	SKOV-3	Ovarian cancer	Cisplatin	Increased percentage of apoptotic cells and G0/G1 cell cycle arrest	[6]
3D Clinostat	SNU-4139S3-TO, SNU-4146S1-TO, SNU-4631AS4-TO	Colorectal cancer organoids	Afatinib, AZD2014, Buparlisib, Irinotecan, Olaparib, MK5108, Oxaliplatin, SAHA and 5-FU	Increased response to 5-FU	[5]

Table 2. Effects of simulated microgravity on the cellular response by chemotherapy.

Simulated Microgravity	Cell Line	Cell Type	Drug	Findings	Reference
RCCS	HL60 K562	Leukemia	Doxorubicin Daunorubicin	Increased cell migration	[2]
RCCS	K562	Leukemia	Hydroxyurea	Inhibition of nucleus/cytoplasm ratio reduction	[3]
RCCS	EPG85-257 RDB, EPG85-257 P	Gastric cancer	Doxorubicin	Decrease cell migration and invasion Cytoskeleton rearrangement	[4]
3D Clinostat	SKOV-3	Ovarian cancer	Cisplatin	Altered cell shape	[6]
3D Clinostat	SNU-4139S3-TO, SNU-4146S1-TO, SNU-4631AS4-TO (colorectal cancer organoids)		Afatinib, AZD2014, Buparlisib, Irinotecan, Olaparib, MK5108, Oxaliplatin, SAHA and 5-FU	Enhanced growth rates	[5]

5. Methodological Considerations and Limitations

Lewis et al. observed that leukemia cell lines (HL60, HL60/AR) exposed to true microgravity during the STS-67 spaceflight exhibited disrupted cytoskeletal organization and impaired drug efflux, resulting in increased intracellular accumulation of doxorubicin [15]. In contrast, Ekpenyong's group found that the same cell line, HL60, when cultured under simulated microgravity using the RCCS, maintained high viability and demonstrated increased migration in response to anthracycline treatment, without significant changes in cell morphology [2]. This divergence is especially noteworthy given the use of the same cell line (HL60) and the same drug (doxorubicin), yet with almost opposite outcomes: increased intracellular retention in actual microgravity versus enhanced migratory capacity in simulated microgravity. Such a comparison between simulated and actual microgravity studies further illustrates the limitations of ground-based models.

Such divergent findings underscore that studying the effects of microgravity on cancer cells entails several significant methodological challenges and limitations that must be carefully considered when designing experiments and interpreting results. Understanding these limitations is crucial for developing robust experimental protocols and drawing valid conclusions from research findings.

Technical Limitations of Microgravity Simulation A primary consideration in microgravity research is the inherent limitations of ground-based simulation systems. The 3D clinostat, while widely used, introduces potential mechanical stress through its rotating mechanism. Despite efforts to minimize shear forces by completely filling

the culture medium, the mechanical stress from rotation may influence experimental results. This creates a challenge in distinguishing between effects purely attributable to microgravity and those resulting from mechanical forces [5]. Researchers must carefully consider these mechanical influences when interpreting their findings and design appropriate control experiments to account for rotation-induced effects.

The RCCS faces similar challenges. While designed to provide a low-shear environment, complete elimination of mechanical stress is virtually impossible. The rotation speed must be carefully optimized for each cell type and experimental condition, as variations in speed can significantly impact cellular responses [3,16].

Experimental Design Challenges Time considerations present significant challenges. The duration of exposure to simulated microgravity must be carefully standardized, as different cellular processes may respond to microgravity conditions over varying time periods [4]. Short-term versus long-term effects may differ substantially, and the optimal duration for observing specific cellular responses must be determined empirically for each experimental system. The issue is reflected in several studies where cancer cellular responses to microgravity (such as changes in viability, cell death mechanisms, and cell cycle arrest) differed notably depending on exposure time [4,6]. Such observations underscore the importance of considering time as a critical variable in experimental design.

Biological Variability and Reproducibility The biological complexity of cancer cells introduces additional layers of variability in microgravity research. Different cancer cell types may respond differently to microgravity conditions, making it difficult to generalize findings across cancer types. Furthermore, the heterogeneity within cancer cell populations can lead to varying responses even within the same experimental group [17].

Control Group Considerations The establishment of appropriate control groups presents unique challenges in microgravity research. While static cultures under normal gravity conditions serve as basic controls, they may not account for all variables introduced by microgravity simulation systems [3]. The need for rotating controls to distinguish between rotation-induced effects and true microgravity effects adds complexity to experimental design and data interpretation.

6. Conclusions

The investigation of microgravity's effects on cancer cells has yielded significant insights into both cancer biology and potential therapeutic strategies, revealing complex cellular responses that could be leveraged for medical advancement. Through research utilizing various simulation platforms, including the RCCS and 3D clinostats, studies have demonstrated that microgravity conditions induce substantial changes in cancer cell behavior, including alterations in proliferation patterns, morphological characteristics, and drug responses. These changes, manifested through modifications in gene expression, cellular signaling pathways, and drug sensitivity mechanisms, suggest promising new approaches for cancer treatment both in space and on Earth. While methodological challenges persist, particularly in simulation technology, the potential of microgravity as a tool in cancer research extends beyond its immediate applications in space medicine. Indeed, observations such as downregulated expression of drug resistance genes in gastric cancer cells (EPG85-257RDB) and cytoskeletal remodeling in ovarian cancer cells (SKOV-3) under simulated microgravity may guide future efforts to overcome chemoresistance in tumors on Earth. The observed enhancement of drug sensitivity in certain cancer types, coupled with modifications in drug resistance mechanisms under microgravity conditions, presents valuable opportunities for therapeutic development. As technology advances and our understanding deepens, the integration of microgravity research into cancer therapeutics may offer innovative strategies for addressing the challenges of cancer treatment, making this field a crucial bridge between space medicine and terrestrial cancer research.

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