

The Role of Microgravity in Cancer: A Dual-edge Sword

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Abstract

Since human beings could travel beyond the earth atmosphere, scientists started to investigate the effect of microgravity on human cells. Microgravity has different effects on normal and cancer cells, but the related mechanisms are not well-known till now. The aim of the present review is to focus on the consequences of exposing the cancer cells to reduced gravity. Some cancer cells organize three-dimensional structures under microgravity. Obviously, microgravity is an external stress, which can affect cell proliferation, apoptosis, cytoskeleton and signaling pathways. In addition, it touches immune-related components, regulates immune responses, and implicates immune cell activation. Low mutation aggregation and cancer rate in astronauts may lead to use microgravity as a therapeutic approach. However, it reduces the invasion and migration in some types of cancer cells, triggers the oncogenic signaling pathways including KRAS, and inhibits proliferation in normal lymphocytes. In conclusion, using microgravity as a therapeutic method in cancer treatment needs to be more investigated on both cancer and normal cells, and might not become true in the near future.

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INTRODUCTION

Gravity is a ubiquitous force, which affects all the creatures on the earth. After man stepping on the moon in 1969, the importance of the gravity became clearer. Astronauts showed various physiological changes under microgravity condition on the space [1]. Initial investigations about the effect of microgravity on human cells were performed through the US Skylab Program within the early 1970s. The aim of these studies was to survey the physiological and biomedical changes through mimicking microgravity on the earth [2]. The results showed that all the biological living systems, whether a complicated organism or single cell, are affected by surrounding environment and adapted to its changes. The human embryonic cells, WI 38 were cultivated in the Skylab III for about 28 days and the results revealed that the growth rate, chromosome banding, mitotic index or cell cycle were similar to human cells on earth [3, 4]. However, there were differences between the Skylab III and on-earth cell cultured media in terms of consuming the components. Raised glucose concentrations in the Skylab III cell-cultured media suggested that micro-

gravity altered cell metabolism. Furthermore, extracellular matrix, cell polarization and cell-cell interaction were different between both Skylab III and on-earth cell-cultured media [5, 6]. Because of the space flights' impacts on astronauts, the study of normal cells under microgravity condition was focused. For instance, to study the effect of space-flights on human cardiocytes, rat neonatal cardiomyocytes were cultured under less than 1g gravity. Protein synthesis of rat neonatal cardiomyocytes decreased under minimal gravity, while apoptosis, cell viability, and protein degradation stayed stable [7]. In an additional study, evaluations showed that microgravity reduced the diabetic-associated genes including HNF1A knockout in the pancreatic cell [8]. In addition to the assessment of the normal cells, some studies focused on the effects of microgravity on cancer cells from different viewpoints. Several biological processes, including apoptosis, cytoskeleton, adhesion/extracellular matrix (ECM), proliferation, cell cycle, DNA repair and DNA replication, stress response, proteolysis, enzyme binding, transcription factor binding, migration, angiogen-

esis, and signal transduction were differentially expressed in cancer cells [9, 10]. The aim of the present review is to focus on the consequences of exposing the cancer cells to reduced gravity. Previously, some studies reviewed related articles till 2013 [11, 12], so the present article is included post-2013 studies. In this review, different effects of microgravity on cancer cell behavior and immune responses are discussed. Some recent studied genes and proteins in cancer cells and their alternation through the reduced gravity are summarized. Finally, the therapeutic application of microgravity in far future is pointed out.

EFFECT OF MICROGRAVITY ON CANCER CELL BEHAVIOR

Viability and Apoptosis

Several studies have shown the inhibitory effects of microgravity on cancer cells viability and growth. Inhibition of an anti-apoptotic protein, BCL-2, and inducing the apoptosis-related proteins, PARP, p-53 and Bax in ml-1 thyroid cancer cells has been demonstrated, through the simulated microgravity condition [10]. The Western blot analysis of BL6-10 melanoma cellular proteins discovered the Caspase-3, 7 and 8 up-regulation and Bnip3 and BCL-2 down-regulation. Moreover, through the reduction of NF- κ B pathway regulating molecules including Uev1A, TICAM, TRAF2, and TRAF6, an apoptosis suppressor complex, NF- κ B/p65 is localized in the cytoplasm [13]. In conclusion, microgravity affects cancer cells by inhibiting survival signaling pathways and inducing programmed cell death. The CAV1 protein is a gravity-sensitive protein, which regulates cellular proliferation, differentiation and apoptosis [14], and decreases in cancer cells after 72h exposing to microgravity [15].

Growth and Proliferation

Studies have indicated that cell cycle regulating proteins like CyclinD1 and B1 were down-regulated under simulated microgravity in breast and colorectal cancers [10, 16]. Recently, Kim *et al.* have studied the effect of microgravity on Hodgkin's lymphoma cancer cells [17] in comparison with normal human dermal fibroblast cells. Interestingly, the proliferation of lymphoma cancer cells was inhibited, and microgravity led to cell death; but normal cells were not affected [17]. ATM/ATR and CDK1/2 proteins are essential for cell cycle transition from S to G2 and decreased under microgravity. The flow-cytometry analysis confirmed that the number of cancer cells in the G2 phase are reduced [13]. Similar findings have been improved in breast and lung cancers [15, 16]. Also, the colony formation assay on cancer cells (melanoma, colorectal and leukemia) revealed that microgravity diminishes the cancer cell ability to form colonies [10, 13]. Briefly, reduced gravity

disturbs cell cycle controlling genes and proteins, so prevents cancer cells from proliferation and forming spherical colonies.

Extra-cellular Matrix and Cytoskeleton

Microgravity can cause some changes in ECM and cytoskeletal proteins in cultured cells and triggers spheroid formation in some types of cancer cells [18]. Spheroids are three-dimensional (3D) cell architecture structures, which can reflect the physiological status of the tumor to some extent [9]. Modifying signaling pathways, the quantity of ECM and 3D growth associated proteins may interact in spheroid formation [18]. Studies on FTC-133 follicular thyroid cancer cell line revealed that the expression of VEGF, EGF and CTGF are up-regulated under microgravity in both two-dimensional (2D) and 3D cultures, but the expression of EGF in 3D is much higher than 2D culture, and the expression of CTGF in 2D is much higher than 3D culture [19, 20]. Both EGF and CTGF implement proliferation and the difference between their expression in 2D and 3D culture shows their different roles through the environment changes. The Ml-1 thyroid cancer spheroids overexpressed intermediate filaments, vimentin and vinculin, in addition to ECM-related proteins, collagen type I and III, laminin, fibronectin, chondroitin sulfate compared with control spheroids [10]. In this regards, mass spectrophotometry analysis indicated that integrin α -5 chains, myosin-10 and filamin B found in FTC-133 cells mediates cell binding to fibronectin and enhances 3D growth [16]. Kopp *et al.* have revealed that the accumulation of F-actin and other cytoskeletal associated proteins toward the breast cancer cells' membrane directed to change the cancer cells' shape and form spheroid [21]. Previously, they had conducted similar study on the thyroid cancer cells and discovered parallel findings [20]. Cytokines, IL-6 and IL-8 regulate integrin beta-1, talin-1, Ki-67, and beta-actin, and mediate the spheroid formation [22]. Another study on thyroid cancer cells confirmed these findings. Bioinformatics analysis revealed that simulated microgravity promoted the overexpression of 3D growth-related proteins and avoided extreme accumulation of exogenous proteins. While structural proteins related genes are up-regulated [18]. After 22 seconds exposing to microgravity, the F-actin and cytokeratin cytoskeleton changed in thyroid cancer cells [23], and PXN, VCL, and PTK2 proteins localized around the focal adhesion complex and led to spheroid formation [24]. As shown in Table 1, ECM and cytoskeleton controlling genes, including *ACTB*, *KRT80*, *OPN*, *FN*, *COL4A5*, *LIMA1*, *TUBB*, and *PFN1* modify under microgravity. It seems that cancer cells remodeled their ECM and cytoskeleton as an adaptive response. To sum up, microgravity causes some changes in cell-cell interactions, cancer cells adhesion, migration and invasion.

Table 1: Microgravity-induced Gene Expression Alteration

Type of Cancer	Cell Line	Type of Cell Culture	Gene	↑	↓	Ref.
Follicular Thyroid Cancer	ml-1	2D	<i>ACTB, KRT80, OPN, FN COL4A5, LIMAI, MTSSI</i>	*	*	[23]
	FTC-133	2D	<i>ACTB, TUBB, PFN-1, ERK2, Casp9, FLT1, FLK1, OSP, CPNE1, TGM2, NGAL, IL-6, IL-8, IL-7, CTGF CAV2</i>	*	*	[9, 15, 19, 20, 38, 39]
3D		<i>ACTB, TUBB, EGF, CTGF, VEGF, ERK1, ERK2, Casp9, FLT1, FLK1, OSP, CPNE1, TGM2, NGAL, IL-6, IL-8, IL-17 CAV1, CAV2, CTGF, PRKCA, ITGB1, MMP3</i>	*	*		
	Nthy-ori3-1	2D	<i>ACTB, TUBB, PFN-1, PKC, ERK1, ERK2, PKB, Casp9, OSP, CPNE1, TGM2, NGAL, Col1A1, IL-6, IL-8, IL-17</i>	*		
		3D	<i>VEGF ACTB, TUBB, PFN-1, PKC, ERK1, ERK2, PKB, Casp9, OSP, CPNE1, TGM2, NGAL, Col1A1, IL-6, IL-8, IL-17</i>	*	*	
	UCLA RO82-W-1	3D	<i>VEGFA, VEGFD, MSN, MMP3 ACTB, ACTA2, KRT8, TUBB, EZR, RDX, PRKCA, CAV1, MMP9, PAII, CTGF, MCP1</i>	*	*	[18]
Breast Cancer	MCF-7	2D	<i>IL8, VEGFA, FLT1, ESR1, PGR1</i>	*		[21]
		3D	<i>ACTB, TUBB, EZR, RDX, FN1, VEGFA, FLK1, Casp9, Casp3, PRKCA</i>		*	
Melanoma Cells	BL6-10	2D	<i>Neil3, Nthl1, PARP, Ung, Brip1, Ercc8, Rad23, Rad51, Xrcc2, Ku70, ATM</i>		*	[13]
Leukemia	DLD-1	2D	<i>CDK1, JUNB, MYC, HEY1 CD117, CDK2, CCNE1, TFRC</i>	*	*	[10]
	HL-60		<i>CDK1, CDK2, CD117, MYC, CD105, CD90 CCNB1, CD71</i>	*	*	
Colorectal Cancer	MOLT-4	2D	<i>JUNB, CD117, CCNB1 CDK1, MYC, ROMO1, CCNE1</i>	*	*	[10]
Lung Cancer	H460	3D	<i>Nanog, OCT-4</i>		*	[15]

↑: Up-regulation, ↓: Down-regulation.

MICROGRAVITY AND IMMUNE SYSTEM RESPONSES

Microgravity results in alternation of immune responses by modifying both immune system's compartments and cells. The effect of microgravity on the compartments of the immune system has been investigated in several studies. As most studied cancer cells under microgravity condition, thyroid cancer cells showed overexpression of inflammatory factors, IL-6, IL-7 and IL-17 proteins. In addition to these molecules, the toll-like receptor downstream protein, NGAL and pro-inflammatory cytokines activated protein, OPN are up-regulated in FTC-133 thyroid cancer cells [20]. Down-regulating the IL I-beta under the reduced gravity condition causes human macrovascular endothelial cells to proliferate and remodel their cytoskeleton faster than the control cells, which expose to normal earth gravity [27]. Researches about the effect of microgravity on immune cells such as mouse's leukocytes in space shuttle showed that mitogenesis of the lymph cells and activity of the cytotoxic T-cells (CTCs) are suppressed. Moreover, CTC released cytokines, which reduced and altered the function and proportions of T-cell subsets. In contrast, certainly accumula-

tion of cytokines in spleen and thymus cells of space-flown rodents were reduced [28, 29]. Other studies on the effect of on-earth microgravity on immune cells illustrated interleukin 1 [24], IL 3, IL 6, tumor necrosis factor, interferon α and γ accumulations in them [30-32]. Under reduced gravity, human monocytic cell line, U937 maintains its capability to differentiate [27], and also down-regulating the IL I-beta caused alternation in the ubiquitous enzyme, protein kinase C distribution, through the cytosol and nucleus of immune cells [33]. In another study, scientists exposed mesenchymal stem cells (MSCs) to the simulated microgravity, and then injected them to the nude mice as an anticancer vaccine. Microgravity-treated MSCs showed increased MHC1 and HSPs proteins expression, and also induced Th1-mediated cytokine and CD8-dependent cytotoxic responses which altogether inhibited proliferation of lung cancer cells and reduced the tumor size and weight [34]. Moreover, the bioinformatics analysis has revealed that the NF- κ B pathway was down-regulated by Notch1 signaling. Then, NF- κ B pathway mediated LPS genes, which played a key role in LPS-stimulated macrophage activation through the microgravity [8].

POSSIBILITY OF A THERAPEUTIC APPROACH

Low mutation aggregation and cancer rate in astronauts [35] may light this idea in someone's mind which microgravity might have the potential to be utilized as a therapeutic approach in cancer treatment. A summary of expression of the altered genes in different cancer cell lines has been mentioned in Table 1. However, our current understanding of microgravity impacts on cancer cells is still limited; there are some controversies among the related studies, which we discussed about them below. Evidently, there is a close crosstalk between cancer treatment and the immune system responses. Most of the immune responses dysregulations may be the cause of cancer occurrence or affect response to therapy [36]. There are some studies that confirm the effects of microgravity on immune cell activation, pro-inflammatory and inflammatory protein accumulation, and inducing cytokines secretion. Furthermore, microgravity inhibits proliferation of the cancer cells and increases the drug sensitivity in B lymphoma, liver, breast and non-small lung cancer [8]. *CTGF*, a connective tissue growth factor gene, is over-expressed in papillary thyroid carcinoma cells under microgravity and has a negative correlation with metastasis, tumor size and clinical stage [37]. Melanoma cancer cells' DNA repair molecules like PARP, Ercc8, Rad23, Rad51, and Ku70 are down-regulated at the levels of transcription and translation. Furthermore, DNA damage response mediating genes, p53, PCNA, ATM/ATR, and CDK1/2 significantly inhibited [13]. Invasion-related genes, *MMP2* and *MMP3* genes and other angiogenesis-related genes in cancer cells declined dependently of gravity [9, 14]. Microarray analysis has discovered that *MIR22HG* as a tumor suppressor gene, 4.4 fold is up-regulated under zero gravity and this finding has been validated by q-PCR. Also, the downstream targets of *MIR22HG*, including *SP1*, *CDK6* and *CCNA2* were dysregulated [10]. In contrast, there are some studies which pointed the role of microgravity in favor of cancer cells. The migratory ability of human lung cancer cell lines of adenocarcinoma and squamous cell carcinoma increased after exposing to microgravity [38]. Researchers have found some evidences that show microgravity may induce some types of cancer like leukemia, lung, breast, ovarian, liver, head and neck cancers. No matter the cancer cells have been cultured on simulated microgravity or 1g gravity, the *KRAS* oncogenic pathway is induced in them [8]. In this regard, cytogenesis results indicate that DNA repair and proliferation-related genes (including *CCND1* and *PCNA*) are down-regulated

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in normal lymphocytes. Also, the expression of apoptotic genes (including *Bax*) was reduced. In conclusion, the accumulation of DNA damaged immune cells may cause malignancy [39]. As shown in Table 1, microgravity has dissimilar impacts on gene expression of various cancer cells. For example, a proto-oncogene, *MYC*, was down-regulated in leukemia and up-regulated in the colorectal cancer. Unlikely, another proto-oncogene, *CD117*, was up-regulated in leukemia and down-regulated in the colorectal cancer [10]. These results show different effects of microgravity on solid and liquid tumors. All in all, it seems too soon to decide about the microgravity therapeutic application in cancer. However, it can be concluded that microgravity alone might induce cancer but along with the other factors like chemotherapy and radiotherapy enhance the impression of the treatment.

CONCLUSIONS

Obviously, cancer cells consider microgravity as an external stress. Over-expression of cellular stress related proteins including HSP70 and ROS, and Ca^{2+} ion evidences this claim [37]. In the microgravity status, some cancer cells assemble to multicellular 3D constructs. Therefore, simulated microgravity may be applies as an inducing factor in tissue engineering and spheroid formation. Microgravity has various effects on the cell growth, proliferation, gene expression, production of soluble factors, cell signaling, ECM production, cytoskeletal organization and cell adhesion. Furthermore, it affects the expression of cytokines, interleukins and other immune-related compartments and activates immune cells. With regard to analytical approaches derived from space-based investigations, it seems that microgravity could be used as an anti-tumor technology and therapeutic method in far future. Therefore, the effects of microgravity on both normal and cancer cells should be more studied.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ETHICS APPROVAL

Not applicable.

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